

The University of Chicago

A STUDY OF SEXUAL
PERIODICITY AND ITS CONTROL
IN THE FEMALE RODENT
CITELLUS

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY

DEPARTMENT OF ZOOLOGY

1934

By
GEORGE FINLAY SIMMONS

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

1937

The University of Chicago

A STUDY OF SEXUAL
PERIODICITY AND ITS CONTROL
IN THE FEMALE RODENT
CITELLUS

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY

DEPARTMENT OF ZOOLOGY
1934

By
GEORGE FINLAY SIMMONS

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS
1937



ACKNOWLEDGMENTS

This problem was suggested to me by Dr. Carl R. Moore, whose constant advice and encouragement have gone far to make the work both pleasant and profitable. I am indebted to Dr. L. V. Domm for permitting me to occupy outdoor space and make use of equipment in the poultry yards of Whitman Laboratory of Experimental Zoology, making it possible for me to maintain fairly large numbers of animals under natural environmental conditions in stock pens, individual hibernation pens, and outdoor cages. The Whitman Laboratory facilities for experimental ecology were kindly made available by Dr. W. C. Allee, so that I was able to conduct light and temperature experiments under the controlled conditions of constant temperature and constant humidity rooms.

Many parts of the experimental phase of this study were possible only through the kindness and courtesy of others in generously providing hormone preparations. I gratefully acknowledge my indebtedness to the following individuals for their cooperation during the course of these experiments: Prof. H. B. Van Dyke and Zonja Wallen-Lawrence, of the Department of Physiological Chemistry and Pharmacology of the University of Chicago, for samples of pituitary and urinary hebin; Dr. Oliver Kamm and Parke, Davis and Company, for preparations of whole sheep pituitary gland extract, antuitrin-S, and theelin; Prof. R. G. Gustavson, of the Department of Physiological Chemistry of The University of Chicago and the University of Denver, for oestrin extracted from pregnancy urine and human placentae; and the staff of the Chicago Lying-In Hospital for pregnancy urine from known periods of pregnancy.



Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41548403_0117

TABLE OF CONTENTS

	Page
LIST OF TABLES.	iv
Chapter	
I. INTRODUCTION	1
II. MATERIAL AND METHODS	3
III. LIFE HISTORY OF THE FEMALE STRIPED GROUND-SQUIRREL	7
IV. OESTRUS CYCLE IN THE GROUND-SQUIRREL	8
V. MORPHOLOGY OF THE FEMALE REPRODUCTIVE ORGANS AND ACCESSORIES	10
VI. CYCLIC CHANGES IN THE FEMALE REPRODUCTIVE ORGANS AND ACCESSORIES	11
VII. EXPERIMENTAL ATTACK ON THE PROBLEM OF PERIODICITY	14
Effects of Bilateral Ovariectomy	
Effects of Administration of Gonad-Stimulating Substances	
Effects of Administration of Oestrogenic Substances	
Assay for Seasonal Variation in Potency of the Anterior Pituitary of the Ground-Squirrel	
Effects of Spaying on the Potency of the Anterior Pituitary of the Ground-Squirrel	
Experimental Analysis of Environmental Factors	
VIII. DISCUSSION	34
IX. SUMMARY AND CONCLUSIONS	40
X. BIBLIOGRAPHY	43

LIST OF TABLES

Table	Page
I. Monthly Distribution of Normal Tissues from Normal Ground-Squirrels.	6
II. Effects of Bilateral Ovariectomy on Reproductive Accessories.	16
III. Reaction to Fresh Anterior Pituitaries from Albino Rats.	18
IV. Reaction to Fresh Anterior Pituitaries from Spayed Guinea Pigs	19
V. Reaction to Injections of Sheep Pituitary Whole Gland Extract.	20
VI. Reaction to Human Pregnancy Urine.	21
VII. Reaction to Gonad ¹ ₂ Stimulating Extracts from Human Pregnancy Urine.	23
VIII. Reaction to Oestrin Application.	24
IX. Sex-Stimulating Potency of Anterior Pituitaries of Female Ground-Squirrels when Implanted into Immature Female Rats.	26
X. Sex-Stimulating Potency of Blood Serum from Female Ground-Squirrels when Injected into Immature Female Rats	28
XI. Effects of Application of Constant Cold (40° F.) and Darkness to Sexually Inactive Adults	32

I. INTRODUCTION¹

The regulation of reproductive periodicity among the vertebrates has long been one of the most interesting and puzzling questions in zoology. The literature of natural history is filled with observations on the cyclic character of the habits of animals in nature, and all evidence points to the profound influence which breeding periodicity exerts on the life of the animal. The reproductive system, instead of being continuously functioning, becomes active at regularly recurring periods. Among vertebrate animals in general these rhythms recur but once each year, the peak usually coinciding with the favorable months of spring. The most conspicuous exceptions occur among domesticated birds and mammals and among laboratory mammals, where the basic cycle in the female may be crowded into a few days or weeks, and repeated at regular intervals many times during the year.

In both birds and mammals, a long series of behavioristic reactions follow each other in the cycle of the year. This is especially well known in birds, where courtship, migration, and nesting phenomena have long been studied and correlated with the calendar. Wild mammals are more difficult to study, hence students are fewer and data more fragmentary.

The fundamental question is that of the mechanism controlling such exact periodicity. This involves the regulation of the length of time between one period of breeding activity and the next. Why will one species of animal breed but once a year--always in April, for example,--and then remain sexually inactive throughout the remainder of the year, while another breeds two or three times during spring and summer, and yet a third species breed practically continuously throughout the year?

What brings on mating activity in mammals, and what stops it if pregnancy does not occur? Is the cycle brought on and stopped by some intricate hormonal interrelationship, or are environmental factors involved? Is the cycle in the female stopped by ovulation or by corpus luteum formation in the ovary? Is the next cycle deferred by persistence in activity of the corpus luteum, or perhaps by lactation and nursing? The questions become

¹This investigation has been aided by a grant from the Committee for Research in Problems of Sex of the National Research Council; grant administered by Prof. F. R. Lillie.

so many and involved that investigations must be confined to a single species where each step in the reproductive cycle may be studied in turn.

Recent attacks on the shorter reproductive cycle have been largely confined to laboratory rodents such as the rat, mouse, guinea pig, and rabbit; to the laboratory carnivore of England, the ferret; to domesticated animals such as the cat, dog, sheep, cow, and horse; and to monkeys. Only scattered observations have been published on native wild species of mammals, unchanged by domestication and still exhibiting the basic type of longer, annual reproductive cycle; such observations, principally in Europe, have usually been made on the male. Among the females of wild mammals, the primitive opossum was early investigated in this country by Hartman, culminating in a series of classic reports (1923-1926), while near relatives were being investigated by Hill and O'Donoghue (1913) and Hill (1918) in Australia. Recently attention has been given to normal processes in the hedgehog by Deanesly (1934) and in the grey squirrel by Deanesly and Parkes (1933).

In this laboratory the male laboratory rodent has received almost exclusive consideration. Observations on the effects of administering gonad hormones and other endocrine substances to the rat and the guinea pig by Moore and Price (1930) suggested a mechanism involving a reciprocal relationship between the gonads and the anterior pituitary gland. A working hypothesis, which has proven of much value in our experimental work, was developed by Moore (1931) and by Moore and Price (1932). At about the same time an almost identical hypothesis for oestrus cycle control was suggested by Brouha and Simonnet (1931).

The confusion of conditions existing in laboratory rodents suggested at once the need for a careful analysis of a seasonal breeding type of mammal where the fundamental pattern still exists. This formed the basis for a project begun in 1931 in this laboratory to investigate normal events throughout the year in both sexes of an annual breeding species and to attempt a further analysis of such events by experimental procedures. The program included a study of the male system by L. J. Wells and of the female system by myself--involving gonads and all reproductive accessories in each sex--while tissues recovered from these animals were used for a cytological study of the pituitary by Dr. Warren O. Nelson, recently National Research Council Fellow in this laboratory and now of the Department of Anatomy, The University of Missouri; the adrenals and thyroids by Dr. Moses Zalesky of this department; and the teeth (especially as indicators of

changes in calcium metabolism) by Dr. Isaac Schour of the Department of Dental Histology of The University of Illinois.

Because of the fact that the most rapid strides in the study of these complex mechanisms are being made in laboratory rodents, the animal chosen for this study is a native wild rodent, the thirteen-lined ground-squirrel or spermophile, Citellus tridecemlineatus (Mitchill). It is a small and weasel-like member of the squirrel family, Sciuridae, and is somewhat smaller and considerably more slender than the average albino rat of the laboratory, hence an excellent species of mammal for study and experimentation. Furthermore, it is sufficiently abundant in the Chicago region so that an adequate number of animals have been available within a range of fifty miles of this laboratory. Certain phases of the reproductive system of this form have recently received attention by Johnson and his students (1931, 1933) at Kansas, and have been continued for a time by one of them, Foster (1934), at Wisconsin. The principal results of my own study on the female ground-squirrel may now be outlined.

II. MATERIAL AND METHODS

Some six hundred females have been used in the present study. They were captured in the field, unharmed, by pouring water down their holes and catching them by hand as they came out. They were carefully examined in the field, marked, and brought in to the laboratory for observation and experimentation. An individual history of each animal was kept at all times, so that the date and place of capture, as well as the state of maturity and sexual condition of the animal at the time of capture, were always known. Stock animals for experimental treatment and laboratory observation were kept in a well-lighted animal room in the basement of Hull Zoological Laboratory, where they lived in large, well-ventilated cages; they were continually supplied with fresh drinking water and with an abundance of mixed grain (corn, wheat, oats, and barley) and pellets of a patented rabbit food containing a so-called balanced diet of vegetable proteins and fats, carbohydrates, and vitamins, while fresh lettuce was furnished at least once a week. Special animals requiring continued observation under outdoor conditions were kept in banks of roofed cages back of Whitman Laboratory, where they were fed as carefully as the caged animals in Hull Laboratory. Still other animals were kept outdoors, back of Whitman Laboratory, in cages measuring approximately ten by eight by eight feet, surrounded and covered by wire mesh, which was also buried to a depth of

about four feet in the ground around each cage or pen; these animals were given mixed grain and fresh water, and also ate the growing corn and cereal grasses sowed in such pens; they dug their own burrows, carried in bedding, and apparently lived as normally as in their native habitat afield.

Animals have been available in the laboratory every month in the year, and were brought in from the field during every month from April to November, inclusive. They were planted in fall in outdoor pens in the country, in the large outdoor pens at Whitman Laboratory, and in small individual hibernating pens at Whitman, constructed by burying a wire-mesh cage (five feet by two by one and a half feet) to a depth of four feet in hard-packed soil, leaving a foot of caged space above ground where food and water were supplied. The animals in these various pens, large and small, dug underground, constructed granary with nest and drain, and hibernated in normal fashion under frozen soil, making it possible to dig animals from burrows at any time during the winter hibernation period.

In the recovery of tissues for morphological and histological study, a standard routine was observed. Each animal was killed with anaesthetic ether, weighed, the ventral surface carefully cleared of hair, and three dimensional measurements taken of the vulva and one nipple (fourth on the left). The presence of a vaginal orifice was noted, and a vaginal smear taken for later staining with haematoxylin and eosin and careful examination; even when the vaginal orifice was closed, a swab could be carefully inserted through the tiny urethra-vaginal connection. The entire ventral surface of the body was cleared to remove all twelve nipples and the complete spread of mammary tissue, which was examined fresh, then the right half floated on physiological saline and fixed with formalin, the left half floated and fixed in Bouin's fluid. The entire reproductive tract was carefully dissected; removed entire to physiological saline; the ovaries, oviducts, uterine horns separated from the vaginal tract, which included the body of the uterus, the cervix, vagina, and vulva; each tissue was then cleared of excess fat, connective tissue or muscle, examined and peculiarities noted, and weighed on a milligram torsion balance. Three dimensional measurements were made of ovaries, uterine horns, and the vaginal tract, with two diameters of the uterine body, cervix, and mid-vagina. A ventral and lateral sketch were made of each ovary, locating corpora lutea, large follicles, or other interesting features to be examined histologically. All tissues were fixed and saved entire, and appropriate parts later cleared in wintergreen or analin-wintergreen, imbedded

in hard paraffin with five per cent rubber, sectioned and stained with Ehrlich's haematoxylin and eosin for histological examination. The whole pituitary was removed, weighed on an analytical balance, and fixed in Zenker's modified aceto-formol solution for study by Nelson. Both adrenals and both thyroids were removed, cleared of excess tissue, measured and weighed individually, and fixed in modified Zenker's fluid for study by Zalesky. The jaws were labeled and fixed in ten per cent formol for Schour's examination. All dissections were made under a powerful dissecting binocular, and all measurements made with a dial micrometer in hundredths of millimeters.

In experimental treatments, operations were performed aseptically under anaesthetic ether. The condition of the reproductive tract was noted, and one ovary, oviduct, and uterine horn usually removed so that the animal served as its own control since compensatory hypertrophy did not occur in short-time treatments. All solutions were injected subcutaneously by hyperdermic, once a day when in oil, two or three times daily in aqueous solutions. Macerated pituitary suspensions were injected intramuscularly, using the hind legs alternately.

Control tissues were well distributed throughout the year. Field and laboratory animals, both adult and immature, were usually killed each month as seasonal controls. Females in from the field as much as a week were classed as laboratory animals, since striking changes sometimes occurred in the reproductive tract within ten days after capture provided animals were kept in the basement animal rooms of Hull Laboratory instead of in the outdoor pens; furthermore, less than two per cent of the females kept through winter and spring in the Hull basements came into full oestrus, others remaining sexually inactive season after season. Hence field animals were usually brought straight in from the field and killed the same day.

Animals were classed as adults only when they had attained or were attaining a period of oestrus or heat, or if they had previously bred; postpartum signs persisted for many months, externally in the continued presence of tiny triangular nipple tabs, resulting from atrophy of last season's nipples and persisting until development of new nipples early the following spring; internally, in the endurance of implantation sites in the uterine horns, showing as dark centers until they are abolished by oestrin activity on the approach of the next oestrus. The resting reproductive tract in adult females never regresses to the low level of such parts in prepubertal females; the adult vulva shows a definite median suture line, marking the previous

TABLE I

MONTHLY DISTRIBUTION OF NORMAL TISSUES FROM NORMAL GROUND-SQUIRRELS

Number of Animals Posted

Months	All Tissues Recovered				Part Tissues Recovered				Totals
	Field		Laboratory		Field		Laboratory		
	Adult	Imm.	Adult	Imm.	Adult	Imm.	Adult	Imm.	
January.....	2	1	1	9	0	0	0	7	20
February....	2	2	2	1	0	0	0	16	23
March.....	3	1	1	2	0	0	0	1	8
April.....	16	0	0	1	0	0	0	2	20
May.....	44	0	2	0	3	0	0	0	49
June.....	37	0	9	0	19	0	0	0	65
July.....	15	3	5	0	10	0	13	0	46
August.....	4	9	2	0	3	1	0	15	34
September...	2	3	2	1	0	0	4	2	14
October.....	0	4	0	13	0	0	0	9	26
November....	1	1	5	2	0	0	0	8	17
December....	2	2	4	1	0	0	0	9	18
Totals....	128	26	34	30	35	1	17	69	340

existence of vaginal introitus; and there are other corroborative though not conclusive differences.

Immature animals, though somatically adult at about three months of age, continued to be classed as immatures until they entered their first oestrus at approximately three months of age. Though of little use in a survey involving conditions in normal field adults, they were found perfect animals for experiments involving stimulation since they were always at a known low level of reproductive activity.

Preceding certain experiments, normal tissues were frequently recovered so that the animal might serve as its own control. The tissues included an ovary, oviduct, and uterine horn, usually from the animal's right side; such animal retained the rest of its reproductive organs to serve as a measure of results attained by experimental treatment. The normal tissues, removed previous to treatment, are indicated in Table I as "part tissues recovered".

III. LIFE HISTORY OF THE FEMALE STRIPED GROUND-SQUIRREL

Female ground-squirrels emerge from hibernation during the early warm days of spring, usually about the third week in April, some ten days later than the males. In mild years they have been observed as early as the first week in April, while after prolonged and severe winters few appear before May 1. A single period of oestrus occurs, lasting only until coitus a few days after emergence--though oestrus in artificially segregated females continues for several weeks. Probably no female in nature escapes impregnation, especially since the males have been above ground long enough to grow strong and active by the time the females, groggy and thin, stagger out of their burrows. Since copulation in this species is normally forced, mating does not depend on or wait on the psychological acceptance reaction of the female; hence many of the females are impregnated while still in prooestrus, with vaginal orifice still practically closed. Gestation occupies four weeks (27 and 28 days), and litters are delivered from mid-May to the first week in June. The young are naked, pink, hairless, blind, and helpless, and number from seven to fourteen with an average of nine. Prenatal counting of the young has shown higher numbers in the uterus than in litters, suggesting resorption of embryos or even death of a few at birth. Lactation commences almost at once, and the young are nursed from twelve nipples for a period of from four to six weeks. At about five weeks of age the young, with eyes open and body protected by a hair coat patterned like that of the mother, are able to follow the mother above ground and feed for themselves, omnivorously--as do the adults in spring and summer--on green grasses, fresh seeds, and a considerable amount of animal food, especially insects and worms, and even occasional lizards and mice. By September 1 the young are apparently fully grown, at that time being as large or even larger than their parents. Food in fall and winter consists practically entirely of weed-seeds and grain; it is principally sunflower and pigweed seeds, with corn, wheat, oats, and barley from surrounding fields. The family breaks up before winter, and the individuals scatter out over the fields.

In fall, the males remain above ground longer than the females; and the young of the year are the last in both sexes to disappear into their holes for hibernation, which usually begins in October in the vicinity of Chicago. The animals remain completely active at 60° Fahrenheit, and begin to show inactivity and numbness at 55°; a few degrees above freezing will usually put the animals into a complete torpor, though if the temperature

drops quickly below the critical point they remain active. Hibernation, however, is not one long sleep as was formerly supposed; it is a series of comparatively short periods, running from a day to several weeks, during which the animal is in a complete cold torpor, its temperature scarcely above that of the environment, and with respiration and heart-beat so slow and light as to be practically imperceptible. Between periods the animal wakes up, becomes warm and active for a few hours or even days, with rapid respiration and heart-beat, and eats some of the dry grain in its winter storeroom underground. During warmer midwinter weather it may even venture above ground, though rarely; and in our outdoor hibernation pens where animals slept several in a burrow and kept each other active and warm, an animal has occasionally been observed running about in eight inches of snow at a temperature of -8° Fahrenheit.

The ground-squirrel is strictly a diurnal animal, active in the day-time, but even then seeking its burrow to escape enemies or rain. Night is spent in the burrow, in spring the animal remaining underground until 9 or 10 o'clock in the morning, and frequently retiring about 4 o'clock in the afternoon. As the days lengthen and grow hotter, the animals are active early in the morning and late in the afternoon, retiring for a siesta below ground between 10 in the morning and 4 in the afternoon; at such times they are found above ground most commonly from 7 to 10 a.m. and from 4 to 7 p.m. Furthermore, the entire dark season, from November to March, is spent in a hibernating burrow below ground, frequently with the entrance to the burrow completely sealed and frozen, so that the animal is quite literally a creature of light and warmth.

IV. OESTRUS CYCLE IN THE GROUND-SQUIRREL

Some six hundred females, both adult and young, have been examined when freshly caught in the field; and their behavior observed from first appearance above ground in early April to last disappearance in early November. Many have been brought into laboratory conditions, or conditions simulating those in the field, and daily examinations made. Vaginal introitus was found to be established during late April or the first few days in May, while complete closure was accomplished by late June or the first week in July.

Vaginal smears were taken on all animals freshly caught, and daily vaginal smears taken on a large number of animals, after the manner first developed by Stockard and Papanicolaou (1917) in

the guinea pig, and later by Long and Evans (1922) in the rat. This sampling of the cell contents of the vagina was found an excellent method for determining the state of sexual activity in the ground-squirrel, since changes in the smear proved even sharper and clearer than in laboratory rodents. Several thousand such smears were carefully stained with haematoxylin and eosin and carefully studied, and the following differences found to be consistent.

Prooestrus.--The prooestral smear, during the approach to breeding activity, is a clear and evenly-distributed one of small, round, nucleated epithelial cells, always occurring individually and never in clumps or sheets; these become larger, cuboidal, as prooestrus progresses, but retain their nuclei. Apparently there is never a puzzling intermixture of other types of cells. In younger animals, approaching their first oestrus, there may be strands ofropy mucous in which the epithelial cells are buried. The vagina is still closed during this period.

Early Oestrus.--Cornification begins, and during a period of from one to three days which corresponds to the true oestrus period of other animals, a complete change is accomplished to a condition where the individual cells are abundant, larger, flatter and somewhat cornified, and yet showing a faint trace of a small nucleus, which soon disappears; no other cell types are present. The vagina is still largely closed, with the split of introitus just beginning at the ventral edge next to the urinary papilla; the vaginal membrane is so thinned in a dorso-ventral line by rapid cell delamination and sloughing within, that it may split wide on coitus, which may even be accomplished without forcing the slit more than a third open.

Late oestrus.--If mating does not occur at an earlier stage, cell proliferation, cornification, and sloughing continues at a rapid rate. The smear consists of a thick paste of large, flat, enucleate cornified cells--unmixed with other types--and extruding from the vaginal orifice, now completely open and exfoliating or everting slightly in moments of excitement. Isolated females remain in this condition for a month or more, during which time mating may be successfully accomplished. In the absence of mating, the few laboratory females which attained oestrus gradually receded or regressed through earlier stages toward a proestral and then an anoestral condition. Outdoor virgin females showed a leucocytic invasion characteristic of the metoestral condition.

Pregnancy.--The smear from pregnant females begins to show leucocytic invasion in about 36 hours after coitus, following which the smear contains an abundance of polymorphonuclear leucocy-

tes, intermixed with cornified wisps and cuboidal epithelial cells. The smear shows considerable mucous, which never occurs during oestrus and only sparsely during anoestrus and early prooestrus.

Parturition and Resorption.--After parturition, or during resorption of embryos in the laboratory, the smear consists of enormous masses of polymorphonuclear leucocytes alone, the latter apparently having cleared out all other cell types and debris by phagocytic action.

Metooestrus.--The period of regression and decline from mating shows a smear containing numerous polymorphonuclear leucocytes, intermixed with regular nucleated epithelial cells and, in the case of unmated females, with scattered cornified wisps or flattened cornified shells apparently containing neither nuclei nor cytoplasm. The typical metooestral smear is like that of pregnancy, but without the strands of mucous; the cells usually occur in abundance.

Early Anoestrus.--Lactating females, as well as those kept from pregnancy and allowed to recede from oestrus through metooestrus, showed a mixed smear consisting of nucleated epithelial cells and polymorphonuclear cells in fair abundance, occurring in a thin colloid-like fluid, occasionally bubbly, frequently contracting slightly away from the epithelial cells on drying, and taking a light eosin stain. The vagina is flaccid, the aperture still open but small; closure occurs toward the end of this period.

Anoestrus.--This is the sparsest sort of smear, characteristic of the period of sexual inactivity covering most of the year, from July to March. It consists of a few small nucleated epithelial cells, occasional monocytes, and traces of mucous. The vaginal orifice begins to close about the middle of June in both lactating females nursing healthy young and in virgin females kept from mating. The edges of the orifice grow together rapidly; if separated artificially, they again quickly grow together. Weekly examination of hundreds of animals in the laboratory and field shows that the orifice remains closed--and the skin grown together--throughout the remainder of the year, from June until the following April.

V. MORPHOLOGY OF THE FEMALE REPRODUCTIVE ORGANS AND ACCESSORIES

In order to determine seasonal changes in the female reproductive organs, as an indication of the seasonal ebb and flow of hormones in the ground-squirrel, it was found necessary first to work out the gross morphology of the system. In general, the reproductive organs of the ground-squirrel are rather like those

of the albino rat. Differences, however, were to be observed.

As in the rat, the ovary of the ground-squirrel lies in a bursa, completely enclosed; but the membrane, instead of fitting closely as in the rat, is quite loose so that the bursa is about three times as long and as wide as the ovary. The infundibular funnel points ventrally in the bursa, away from the ovary; and the oviduct itself extends and contracts with changes in the annual oestral cycle, so that during anoestrus the ovary is considerably removed from the anterior end of the uterine horn while during oestrus it is drawn close against the anterior end, apparently due largely to contraction of smooth muscle in the free edge of the broad ligament lying medio-ventral to the loops of the oviduct. The body of the uterus shows quite distinctly a bipartite formation, and the cervical region is larger and firmer than in the rat; the lumen of each uterine horn maintains its individuality through both the body of the uterus and the cervix, opening laterally from the vaginal process of the uterus into the upper end of the vagina; this is important in that it prevents transference or migration of fertilized ova from one horn of the uterus to the other.

The inactive ovary of the ground-squirrel is shaped like that of the prepubertal rat. In the sexually active ground-squirrel, the form of the ovary is usually quite regular until late in pregnancy when the corpora lutea begin to bulge hugely from its surface; even here the structure is simple, however, because only one crop of corpora lutea is present--those of previous pregnancies or oestral having long since been completely abolished. In the sexually active rat, on the other hand, numerous successive crops of corpora lutea are present in confusion at one time, so that any shape or size may be assumed.

Of great importance in operative treatment is the fact that in the ground-squirrel, unlike the rat, the broad ligament is free at its anterior end, with the blood-supply entering entirely posteriorly. Removal or examination of the ovaries and uterine horns is thus made easier than in the rat and vastly easier than in the guinea pig.

VI. CYCLIC CHANGES IN THE FEMALE REPRODUCTIVE ORGANS AND ACCESSORIES

The striking morphological changes found from season to season in the ovary and reproductive accessories are accompanied by equally conspicuous histological changes.

Ovary.--The ovary during the inactive season is small,

measuring about 3.5x2.5x1.5 mm., and weighing 4 to 6 mg. The follicles are tiny and confined to the medullary portion, all showing atresia. During mid-hibernation numerous small atretic follicles are still present, but from early December all through the winter ten or fifteen follicles in each ovary are seen to be larger in size and are present in the cortical region, until by late January they are beginning to bulge slightly from the surface, while at the time of emergence from hibernation in April the bulging surface follicles are conspicuous though still rather small and the ovaries measure 3.7x2.7x1.7 mm. and weigh 4 to 9 mg. By the time of oestrus the follicles have enlarged to 0.5 mm., thickening and filling out the ovary to an occasional 4.0x3.0x2.0 mm., with a weight of 10 to 14 mg.

In the ground-squirrel ovulation normally occurs only after coitus, the time interval between the two varying considerably in different individuals, with apparently 12 and 60 hours as the limits. The ruptured follicles immediately set about reorganization and by the fourth day the corpora lutea are well formed, though still remaining principally as internal ovarian structures due to the continued follicular activity of the ovary throughout the course of pregnancy; large follicles continue to bulge from the surface of the ovary, among the corpora lutea, until after parturition, when follicular activity ceases and atresia attacks all small follicles. The stroma of the ovary regresses during lactation, leaving the huge corpora bulging from the surface of the ovary so that it may now resemble a cluster of grapes. The corpora lutea remain large and apparently active until the end of July, when regression sets in and is practically completed by September 1.

The heaviest ovaries were found during pregnancy and weighed from 10 to 24 mg., with an average of 14 mg. Postpartum ovaries averaged between 10 and 12 mg., due to stromal and follicular atresia; yet they were actually larger than pregnant ovaries because of overgrowth and bulging of the part of the corpora lutea.

Oviduct.--The oviduct during anoestrus is slender and light in weight, averaging only 4 to 6 mg., with low epithelium which doubles its height by the time of oestrus, when a weight of 10 to 14 mg. is attained. The heaviest oviducts were found during pregnancy, when a weight of as much as 22 mg. was attained, though the average was nearer 14 mg; the epithelium is lower than during oestrus, but remains higher than in the anoestral condition.

Uterine Horn.--During anoestrus the uterine horn is small,

slender, inactive, and flaccid. Its endometrium is low, with few uterine glands and these very small and slight. A single horn weighs approximately 40 to 60 mg. By the end of March it weighs 80 to 100 mg. or more, and the muscle and endometrial layers are beginning to thicken and glands are beginning to push in from the epithelial lining. By the time of oestrus the horn weighs from 300 to 1200 mg. and contains high epithelium and long straight glands. During prooestrus and oestrus the uterus shows spontaneous motility, which increases to oestrus, when striking shortening, sinuous, and peristaltic movements are observed both in situ and saline. The glands become enlarged after copulation and ovulation, regressing toward the end of pregnancy; parturition brings on the wholesale changes of regression, which are practically completed by the end of lactation, when the horn is once more below 100 mg. in weight.

Vaginal Tract.--During anoestrus the vulva is low and soft, the entire tract small, slender, and firmly soft like ordinary muscular tissue. The weight of the entire tract, including body of the uterus and cervix, is about 300 mg. or less. The orifice is closed and the lumen of the tract is lined with a thin cuboidal epithelium. During hibernation in winter the vulva begins to enlarge, swell, and turn pink; cell proliferation causes the vaginal lumen to become definite and the entire tract to thicken markedly and to lengthen somewhat, so that by early April and prooestrus the tract weighs from 300 to 600 mg. Cornification, especially in the region of the cervix and upper vagina, goes on rapidly so that those sections appear enlarged, hardened, and whitened externally. As in the rat, sloughing of cells lining the lumen becomes more rapid, giving the characteristic smears of early and late oestrus. Cell proliferation drops off and a striking leucocytic invasion occurs several days after coitus. Following the establishment of pregnancy the tract undergoes gradual regression, especially after parturition and its ensuing sweep of leucocytes, until by early July the weight is once more down about 300 mg., the epithelial lining low, the vulva closed and small with little swelling exteriorly, and the cervical region small and flaccid once more.

Nipples.--On the adult animal which has experienced a previous pregnancy, the locating of the twelve nipples during the anoestral period can be determined by looking for tiny black palps measuring about 0.5x0.8x1.15 mm., standing over an almost imperceptible wrinkle or slight flesh-colored mound measuring perhaps 1 mm. in its three dimensions. The palps are the shrunken nipples of the previous pregnancy, and are very gradually rubbed

off until they are lost entirely at about the time of the next pregnancy. A new nipple is built up from the surrounding skin, swelling slightly until at the time of oestrus it measures about 1.9x2.0x1.0 mm. in length and width of base and height from surrounding skin. Nipples widen and fatten slightly during mid-pregnancy, with a height of 1.60 to 2.0 mm. when the vesicular lumps in the uterus are about 10 mm. long; in a few days the nipples enlarge markedly, so that by the time the lumps in the uterus are 25 mm. long the nipples measure 2.5x3.0x3.0-5.0 mm., which size is maintained until lactation gets under way. During mid-lactation, the nipple frequently swells wider, darkens to blackish, loses most of its hairs, and is pulled longer by the hungry young. After lactation, the nipple becomes flattened and reflected posteriorly, hardening and shrinking as it is pressed back against the skin, blackening and decreasing in size until it becomes the tiny palp of the following fall and winter.

Mammary Glands.--There is absolutely no spread of mammary tissue during anoestrus, at which time the only potential tissue is a tiny lump immediately under the nipple and rarely measuring more than 2 mm. across. There is a slight spread of primary tubules during oestrus, but even after coitus a thin spread covers only an area having both diameters 4.5 mm. across. It is almost impossible until after mid-pregnancy to detect the spread of the gland without staining the few delicate little tubules. By the time the vesicular lumps in the uterus are 18mm. across, though they are still so thinly distributed as to make detection difficult. By the time the vesicles are 25 mm. long, the spread is practically complete, though still very thin. The fourth gland on the left, consistently studied as average and typical, has an approximate spread of 20-25 mm. antero-posteriorly and 30 mm. wide from center-line to flank; exact measurement is difficult because of the complete fusion of all glands medially and antero-posteriorly when full spread is attained. Such fourth gland has a thickness of about 0.75 mm. in a pregnant female containing 38 mm. vesicles, at term; 2.3 mm. immediately after parturition; 3.3 mm. in late June in a female lactating abundantly; and down to 1.0 mm. during the first week in July. By July 15 the gland has receded to a tiny lump under the base of each nipple, with no apparent spread or activity.

VII. EXPERIMENTAL ATTACK ON THE PROBLEM OF PERIODICITY

Various types of experimentation have been employed to analyze the factors controlling this more or less exact succession

of events in reproductive activity, some requiring further report in greater detail than is needed here to demonstrate essential relationships.

1. Effects of Bilateral Ovariectomy

Despite the apparent state of inactivity of the ovary during anoestrus, and the regressed and involuted condition of the accessory reproductive organs during this period of breeding inactivity, it was found that removal of both ovaries in the female ground-squirrel induced still further changes. Some of these are interpreted as slightly greater states of involution, while others seem the attainment of a general somatic balance not found in animals going through cyclic activity. Twenty-five animals were kept in a spayed condition for periods ranging from 25 to 94 days; six others were also kept in the laboratory for 337 to 459 days after spaying; and several others were spayed and placed outdoors, where they overwintered below ground in normal hibernation. The majority of these showed greater involution of uterine horns and vaginal tract as indicated by weight, as indicated by typical examples given in Table II, though a single specimen No. 574 had tissues larger and heavier than in unoperated females. This female agreed with all other spayed females, however, in differing histologically from normal anoestral females in having the furrows between the vaginal rugae practically completely obliterated, yet indicated by tree-like clusters of prominent nuclei extending into a less extensive sub-mucous layer; and in having a much lower endometrium with practically no glands, but with occasional glandular cysts; both uterus and vagina, even in the long-time spayed females, are lined by a well-organized though low columnar epithelium, whereas the linings in anoestral females show much greater irregularity and poorer organization until oestrin begins to build them up again. These histological peculiarities and differences are not clearly understood and require further clarification.

These spaying changes developed whether bilateral ovariectomy was performed in early spring, in early summer after parturition, or in fall. In postpartum animals, whose uterine horns carried the small, white-capped, dark-centered lumps marking the sites of implantations, these lumps persisted even throughout the long period of spaying, and were still present at autopsy more than a year after spaying. This is understandable in view of the fact that such points persist in the normal adult female through summer and fall, being gradually abolished in midwinter

TABLE II

EFFECTS OF BILATERAL OVARECTOMY ON REPRODUCTIVE ACCESSORIES

No.	Operated 205	Operated 215	Control 271	Operated ₁ 574	Operated 584	Operated 586
Age	Adult	Adult	Adult	Adult	Adult	Adult
Previously	Pregnant	Pregnant	Pregnant	Pregnant	Virgin	Pregnant
Source	Lab.	Lab.	Field-Lab.	Lab.	Lab.	Lab.
Condition	Regressed	Regressed	Regressed	Regressed	Receded	Regressed
Spayed	Aug. 1	Aug. 12	No	July 17	July 16	July 21
Period	75 days	61 days	-----	337 days	322 days	339 days
Kept	Lab.	Lab.	Lab, 51 d.	Lab.	Outdoors	Lab.
Posted	Oct. 15	Oct. 12	Nov. 22	June 19	June 3	June 25
Uter. Horns						
R., Weight	.0304	.0341	.0406	.0618	.0220	.0456
Diams.	1.20x0.90 ₂	1.00x0.80	1.10x0.65	1.50x0.90	0.95x0.50	1.22x0.67
Cond.	Quite low	Quite low	Low	Low	Quite low	Low
L., Weight	.0283	.0291	.0335	.0413	.0258	.0381
Diams.	0.95x0.85	1.10x0.80	1.10x0.65	1.45x0.78	1.12x0.68	1.17x0.68
Cond.	Quite low	Quite low	Low	Low	Quite low	Low
Vagina						
Orifice	Closed	Closed	Closed	Closed	Closed	Closed
Smear	Sparse An.	Sparse An.	Ancestral	Sparse An.	Sparse An.	Sparse An.
Condition	Very low	Very low	Low	Quite low	Quite low	Quite low
Weight	.2210	.1414	.2938	.4113	.2720	.2296

₁Tissues of No. 574 larger and heavier than in other spayed females, but no signs of regenerating ovarian tissue could be found and, histologically, the accessories resembled those of other spayed females.

₂Long and threadlike in situ, the uterine horns of No. 205 became very short by contraction of longitudinal muscles during fixation.

as the ovary and tract become active once more; the last of such lumps were observed in a hibernating female in February.

This involution and the histological changes following spaying indicate that even during the period of apparent sexual inactivity the ovaries exert at least a minimal effect on the reproductive accessories.

Unilateral ovariectomy, commonly practiced in these experiments to make the animal serve as its own control, was performed on a number of animals otherwise untreated. Such semi-spayed animals kept in the laboratory remained sexually inactive through the following winter, spring, and summer, as did the large majority of apparently normal laboratory females still possessing both ovaries. Several kept outdoors through the winter in hibernation pens and cages came into sexual activity slightly ahead of their unoperated fellows, but this may be due to less total hibernation on the part of such semi-spayed individuals since one which was under daily observation in an above-ground

cage outdoors was found to be hibernating for a few hours on each of only three days throughout the winter season. At least we can say that there is no conspicuous compensatory hypertrophy to be observed in the present species, especially in shorter-time experiments.

2. Effects of Administration of Gonad-Stimulating Substances

It has been demonstrated in a number of vertebrate forms that the gonad is not a self-regulating mechanism but is under the control of the anterior pituitary gland or hypophysis and of similar substances apparently elaborated by the placenta during pregnancy. A series of experiments were carried out at different times of the year to demonstrate that the ovary and reproductive accessories of the ground-squirrel react in like manner to such gonad-stimulating substances.

Fresh Anterior Pituitary Implants.--I obtained excellent results by using Smith's (1926) technique. It consists, briefly, in a quick and careful dissection of a donor animal--here I used guinea pigs and albino rats in different known states of sexual activity or inactivity--with removal of the anterior pituitary, which was macerated in a drop of physiological saline and injected directly into the muscles of the animal in which stimulation was sought. Female ground-squirrels were treated with rat anterior pituitaries in January, March, April, July, and August; and with guinea pig anterior pituitaries in May and December. I was able to induce good stimulation, as shown by very great increases in weight of the affected structures and by striking changes in the histological picture. Treated ovaries became much larger and markedly heavier, as may be seen by reference to Tables III and IV. The rat anterior pituitaries were much more potent than those from guinea pigs, even though the latter were much larger. One rat anterior pituitary each day was too heavy a dosage, since the small internal follicles were not only enlarged and pushed to and beyond the surface of the ovary, with abundant production of oestrin as shown by uterine and vaginal reaction, but they were also overstimulated into huge blood follicles by too rapid enlargement and capillary rupture. Control uteri showed a low and relatively inactive endometrium, while treated uteri had a very high and active endometrium lined by high columnar epithelial cells. A similar reaction occurred in the vagina, where untreated animals showed great thickening of the submucosa, with frequent enormous thickening and cornification of the epithelial lining. Such stimulation was secured, even during the period of regression

TABLE III

REACTION TO FRESH ANTERIOR PITUITARIES FROM ALBINO RATS

Animal	Treated	Treated	Treated	Treated	Treated	Control
No.	50	231	511	513	653	659
Age	Ad. imm.	Adult	Adult	Ad. imm.	Yng. imm.	Yng. imm.
Source	Lab.	Lab.	Lab.	Lab.	Field	Field
Condition	Low Prepu.	Regressing	Procest.	Prepub.	Prepub.	Prepub.
Operated	Jan. 8	Aug. 18	Feb. 24	Feb. 24	No	No
Spaying	Unilat.	Unilat.	Unilat.	Unilat.	None	None
Daily dose	2 a.p. ₁	1 a.p.	1 a.p.	0.5 a.p.	1 a.p.	None
Donor	Ad. ♀♀	Ad. ♂♂	Ad. ♂♂	Ad. ♂♂	Yng. ad. ♀♀	None
Period	14 days	5 days	4 days	4 days	5 days	None
Total dose	0.1540	0.0535	0.0307	0.0177	0.0430	None
Begun	Jan. 8	Aug. 22	Mar. 13	Mar. 13	July 23	
Posted	Jan. 22	Aug. 29	Mar. 18	Mar. 18	July 29	Aug. 2
O.R., char.	Untreated	Untreated	Untreated	Untreated	Treated	Untreated
Weight	.0026	.0094	.0036	.0037	.0113	.0057
Foll.	Tiny, few	None	Med. com.	Med. few	Large, few	Tiny, few
Posit.	Internal		Subsurf.	At surf.	External	Internal
C.L.	None	ca. 5 old	None	None	5 big b.f.	None
O.L., char.	Treated	Treated	Untreated	Treated	Treated	Untreated
Weight	.0227	.0333	.0034	.0257	.0095	.0042
Foll.	Huge, few	Large, com.	Tiny, few	Tiny, few	Large, few	Tiny, few
Posit.	External	External	Internal	Internal	External	Internal
C.L.	B.f. only	19 b.f.	None	17 b.f.	4+ b.f.	None
Uter. Horns						
R., char.	Untreated	Untreated	Untreated	Untreated	Treated	Untreated
Weight	.0310	.0457	.1303	.0607	.2104	.0081
Situ	1.00x1.00	1.70x0.90	2.15x1.75	1.30x1.10	2.75x2.40	1.40x0.60
Condit.	Inactive	Regressing	Prac. inact.	Inactive	Vasc., mot.	Inactive
L., char.	Treated	Treated	Treated	Treated	Treated	Untreated
Weight	.2041	.2918	.0301	.1514	.2042	.0061
Situ	2.40x2.20	3.50x3.30	0.80x0.60	2.28x2.20	2.70x2.35	1.30x0.70
Condit.	Vasc., mot.	Vasc., mot.	Inactive	Med. mot.	Vasc., mot.	Inactive
Endomet.	High	High	Low	Med. high	Very high	Low
Vagina						
Orifice	Thinning	Aug. 29	Closed	Thinning	July 28	Closed
Smear	Procest.	Abun. E	Ancest.	Abun. E	Abun. E	Prepub.
Corn. cond.	Low act.	High	Inactive ₂	High act. ₂		Inactive
Weight		0.7845			0.4600	0.1110

₁Every other day for 8 days, then every day for 6 days, subcutaneously in this female, No. 50.

₂Operative inspection only, animal not killed.

and inactivity (see especially female No. 231 in Table III). The degree of stimulation depended, of course, on the amount and potency of the anterior pituitary material injected. That this stimulation acted through the ovaries of the female ground-squirrels is shown by female No. 511 in Table III, treatment following the removal of both ovaries and giving no suggestion of stimulation in uterus or vagina.

TABLE IV

REACTION TO FRESH ANTERIOR PITUITARIES FROM SPAYED GUINEA PIGS

Animal No.	Treated 283	Treated 284	Treated 285	Treated 286	Treated 287	Control 290
Age	Ad.imm.	Ad.imm.	Ad.imm.	Ad.imm.	Ad.imm.	Ad.imm.
Source	Lab.	Lab.	Lab.	Lab.	Lab.	Lab.
Condition	Prepub.	Prepub.	Prepub.	Prepub.	Prepub.	Prepub.
Operated	Nov. 30	Nov. 30	Dec. 2	Dec. 2	Dec. 2	Dec. 2
Spaying	Unilat.	Unilat.	Unilat.	Unilat.	Unilat.	Unilat.
Daily dose	1 a.p.	1 a.p.	1 a.p.	1 a.p.	1 a.p.	None
Period	4 days	1 day ₁	5 days	1 day	4 days ₁	None
Total dose	.0404	.0116	.0472	.0118	.0393	None
Posted	Dec. 10	Dec. 10	Dec. 10	Dec. 10	Dec. 10	Dec. 12
Ovaries						
R.,Untreat.						
Weight	.0034	.0034	.0043	.0040	.0047	.0043
Foll.	Tiny,few	Tiny,few	Tiny,few	Tiny,few	Tiny,few	Tiny,few
Position	Internal	Internal	Internal	Internal	Internal	Internal
C.L.	None	None	None	None	None	None
L.,Treated						Untreated
Weight	.0053	.0052	.0073	.0057	.0088	.0029
Foll.	Large,few	Large,few	Large,few	Large,few	Large,few	Tiny,few
Position	Surface	Subsurf.	Surface	Subsurf.	Surface	Internal
C.L.	None	None	None	None	None	None
Uter,Horns						
R.,Untreat.						
Weight	.0212	.0196	.0116	.0140	.0125	.0138
Situ	1.00x0.70	1.00x0.70	0.80x0.70	1.00x0.70	1.00x0.80	0.90x0.75
Condit.	Low inact.	Low inact.	Low inact.	Low inact.	Low inact.	Low inact.
Endomet.	Low	Low	Low	Low	Low	Low
L.,Treated						Untreated
Weight	.2224	.0541	.3723	.0164	.1932	.0093
Situ	3.00x2.80	1.60x1.40	3.90x3.40	1.90x1.50	3.25x3.00	1.00x0.70
Condit.	Vasc.,mot.	Vasc.,mot.	Vasc.,mot.	Inactive	Vasc.,mot.	Inactive
Endomet.	High	Mod.high	High	Prac.low	Very high	Very low
Vagina						
Orifice	Thinned	Closed	Nearly	Closed	Thinned	Closed
Smear	Sparse E	Early Pro.	Early E	Early Pro.	Pro.+E	Prepub.
Corn.Cond.	Fair high	Beginning	Very high	None yet	Med.high	Low,inact.
Weight	.4556	.2585	.5938	.2725	.3094	.1890

₁One day interval between last implantation and posting in Nos. 284 and 287, no interval in others.

Sheep Pituitary Whole Gland Extracts.--Eleven female ground-squirrels were given injections of sheep pituitary substance (Parke, Davis and Company) in April, June, July, August and December. Excellent stimulation was quickly secured, the ovary frequently swelling with large follicles which became huge blue-black blood follicles in higher dosages; the uterus was swollen and filled with uterine glands and showed fine motility; the vaginal orifice appeared and the vagina gave a full oestral smear,

TABLE V

REACTION TO INJECTIONS OF SHEEP PITUITARY WHOLE GLAND EXTRACT

Animal No.	Treated 133	Treated 184	Treated 189	Control 871	Treated 657	Control 659
Source	Lab.	Lab.	Field	Field	Field	Field
Age	Ad.virg.	Ad.litt.	Ad.litt.	Ad.litt.	Yng.imm.	Yng.imm.
Condition	Receded	Regressed	Regressed	Regressed	Prepub.	Prepub.
Operated	No	No	July 20	No	No	No
Spaying	None	None	Bilateral	None	None	None
Treatment	Pit.ext.	Pit.ext.	Pit.ext.	None	Pit.ext.	None
Daily dose	1 cc.	1 cc.	1 cc.	None	0.2 cc.	None
Period	10 days	9 days	11 days		5 days	
Posted	July 9	July 6	Aug. 1	July 12	July 30	Aug. 2
Ovaries	Treated	Treated	Untreated	Untreated	Treated	Untreated
R.,Weight	.1396	.0728	.0110	.0080	.0097	.0057
Position	External	External	Internal	Most.int.	External	Internal
Foll.	Many,big	Many,big	Tiny,few	Tiny,few	Many,med.	Tiny,few
C.L.	ca.30 b.f.	ca.16 b.f.	5 old	3 old	16 b.f.	None
L.,Weight	.1425	.0684	.0125	.0210	.0099	.0042
Foll.	Many,big	Many,big	Tiny,few	Tiny,few	Many,med.	Tiny,few
Position	External	External	Internal	Internal	External	Internal
C.L.	ca.30 b.f.	ca.16 b.f.	7 old	10 old	16 b.f.	None
Uter.,Horns	Treated	Treated	Treated	Untreated	Treated	Untreated
R.,Weight	.6119	.5077	.0551	.0555	.1396	.0081
Situ	4.00x3.80	3.20x3.00	1.50x0.60	1.40x0.80	2.90x2.60	1.30x0.70
Condit.	Vasc.,mot.	Vasc.,mot.	Inactive	Inactive	Vasc.,mot.	Inactive
Endomet.	High	High	Low	Low	Mod.high	Low
L.,Weight	.6491	.4865	.0620	.0582	.1324	.0061
Situ	3.90x3.60	3.20x3.00	1.20x0.60	1.70x0.60	3.00x2.70	1.40x0.60
Condit.	Vasc.,mot.	Vasc.,mot.	Inactive	Inactive	Vasc.,mot.	Inactive
Endomet.	High	High	Low	Low	Mod.high	Low
Vagina						
Orifice	June 30	July 2	Closed	Closed	July 29	Closed
Smear	Pro.+E	Pro.+leu.	Anoest.	Anoest.	Abun. E	Prepub.
Corn.cond.	High	High	Low inact.	Low inact.	Mod.high	Low inact.
Weight	.7555	.8345	.2205	.2818	.2427	.1110

exhibiting characteristic epithelial cornification like that found during late April in nature. Sheep pituitary powder preparations (hebin) were tried in two females in January and similar satisfactory results obtained.

Pregnancy Urine.--Raw urine from pregnant women, which perhaps contained some female sex hormone in addition to the gonad-stimulating, anterior-pituitary-like substance attributed to the placenta, was used in four female ground-squirrels in November and January. These animals were in the characteristically low state of reproductive activity found in somatically adult immatures, but they responded quickly to stimulation. Treated ovaries showed marked enlargement, with rapid follicular growth, until under longer treatments they were usually bulging with a few large cystic

TABLE VI

REACTION TO HUMAN PREGNANCY URINE

Animal No.	Treated 12	Treated 29	Control 30	Treated 267	Control 268	Treated 49
Source	Lab.	Lab.	Lab.	Lab.	Lab.	Lab.
Age	Ad.imm.	Ad.imm.	Ad.imm.	Ad.imm.	Ad.imm.	Ad.imm.
Condition	Prepub.low	Prepub.low	Prepub.low	Prepub.low	Prepub.low	Prepub.low
Operated	Oct. 5	Oct. 27	Oct. 27	No	No	Dec. 15
Spaying	Unilat.	Unilat.	Unilat.	None	None	Unilat.
Treatment	Urine	Urine	Phys.sal.	Urine	None	Urine
Preg.stage	8 mo.	4 mo.	None	7-8 mos.	None	7-8 mos.
Daily dose	2 cc.	1.5 cc.	1 cc.	2 cc.	None	1.5-3 cc.
Period	28 days	19 days	19 days	55 days	None	31 days
Posted	Nov. 23	Nov. 18	Nov. 18	Nov. 21	Nov. 21	Jan. 16
Ovaries						
R.,Cond.	Untreated	Untreated	Untreated	Treated	Untreated	Untreated
Weight		0.0064	0.0025	0.0478	0.0055	0.0067
Foll.	Few,tiny	Few,tiny	Few,tiny	Big,com.	Few,small	Few,small
Position	Internal	Internal	Internal	External	Internal	Internal
C.L.	None	None	None	None	None	None
L.,Cond.	Treated	Treated	Untreated	Treated	Untreated	Treated
Weight	.1548	.0811	.0027	.0345	.0047	.0152
Foll.	Cyst.big	Big, few	Small,few	Large,com.	Small,few	Prac.none
Position	Surface	Surface	Internal	External	Internal	Internal
B.F.	None	Few,big				
C.L.	Few,oblit.	Few,oblit.	None		None	Many,big.
Uter.Horns						
R.,Cond.	Untreated	Untreated	Untreated	Treated	Untreated	Untreated
Weight	.0050	.0213	.0255	.3929	.0151	.0575
Situ	1.0	1.2	1.0	4.2	0.9	1.2
Activity	None	None	None	Vasc.,mot.	None	None
L.,Cond.	Treated	Treated	Untreated	Treated	Untreated	Treated
Weight	.4400	.1734	.0215	.3826	.0178	.5385
Situ	4.1	3.9	1.0	4.5	0.9	4.9
Activity	Vasc.,mot.	Vasc.,mot.	None	Vasc.,mot.	None	Vasc.mot.
Endomet.	High	Mod,high	Low	High	Low	Very high
Vagina						
Orifice	Oct. 9	Nov. 7	Closed	Oct. 2	Closed	Dec. 21
Smear	Abun. E	Abun.E+leu.	Prepub.	Com.E+leu.	Prepub.	Abun. E
Corn.Cond.	High	Low	Low	Approach.	Low	High
Weight				.6382	.0928	

follicles, between which are seen a few small corpora lutea in process of being obliterated by the bulging cystic follicles. In one case, however, female No. 49 in Table VI, having approximately the same treatment with the same type of urine given female No. 12, the ovary contains large numbers of prominent corpora lutea with practically no follicles; it appears as if follicular enlargement had occurred, followed by leutinization in a normal manner, without over-stimulation of follicles to become cystic. Uterine horns enlarged markedly under treatment, become highly vascular,

showed strong motility, with high endometrial development showing good development of high columnar epithelium surrounding the lumen. The vaginal tract showed marked enlargement and increase in weight, with increase in thickness of submucosal tissues and striking cornification of vaginal epithelium, the latter showing a tendency to return to a lower condition under continued treatment--hence the smear changed from that of full oestrus to a peculiar mixed smear of nucleated epithelial cells and completely cornified cells, the latter appearing and disappearing in the smear at different times during treatment. The action of pregnancy urine was, therefore, not unlike that of fresh anterior pituitaries, sheep pituitary extract, and pregnancy urine extracts; no consistent differences appeared.

Extracts of Pregnancy Urine.--Sixteen animals were treated with purified extracts of gonad-stimulating substance (urinary hebin and antuitrin-S) from human pregnancy urine. Treatments were given in March, July, August, November, and December, and satisfactory stimulation of ovaries and reproductive accessories was secured (See Table VII); this was indicated by a marked increase in weight of stimulated organs and tissues, though antuitrin-S showed much less effect on reproductive accessories in spite of considerably greater stimulation of treated ovaries. There was no overstimulation of follicles by urinary hebin, and the ovaries gave a fairly normal spring-time picture; antuitrin-S, on the other hand, caused great enlargement of a fair crop of follicles, which quickly turned into magenta, maroon, and black blood-follicles. Both extracts induced the same enlargement and stimulation of uterus and vagina, though that of urinary hebin was slightly greater as previously mentioned; all vaginas showed marked enlargement of sub-mucosal areas, with moderately high to very high cornification resulting in a full and abundant oestral smear accompanied by establishment of vaginal introitus. In uterine horns of treated animals, there was a strong enlargement of the endometrium, with increase abundance of uterine glands, accompanied by the establishment of a high columnar type of epithelium lining the lumen. Striking motility was also induced, making it difficult to complete dissections of freshly removed tissues placed in physiological saline because of twisting, coiling, sinuous, and peristaltic movements in many of these tissues. Such extracts, therefore, proved excellent gonad stimulants.

3. Effects of Administration of Oestrogenic Substances

The injection of the ovarian hormone, oestrin (or theelin),

TABLE VII

REACTION TO GONAD-STIMULATING EXTRACTS FROM HUMAN PREGNANCY URINE

Animal No.	Treated 23	Treated 24	Control 30	Treated 277	Treated 282	Treated 279
Source	Lab.	Lab.	Lab.	Lab.	Lab.	Lab.
Age	Ad.imm.	Ad.imm.	Ad.imm.	Ad.imm.	Ad.imm.	Ad.imm.
Condition	Prepub.low	Prepub.low	Prepub.low	Prepub.low	Prepub.low	Prepub.low
Operated	Oct. 27	Oct. 27	Oct. 27	Nov. 30	Nov. 30	Nov. 30
Spaying	Unilat.	Unilat.	Unilat.	Unilat.	Unilat.	Unilat.
Treatment	Hebin	Hebin	Phys.sal.	Antuitrin	Antuitrin	Antuitrin
Daily dose	1 cc.	1 cc.	1 cc.	5 R.U.	10 R.U.	25 R.U.
Period	19 days	19 days	19 days	7 days	7 days	7 days
Posted	Nov. 18	Nov. 18	Nov. 18	Dec. 12	Dec. 12	Dec. 12
Ovaries						
R.,Untreat.						
Weight	0.0020	0.0018	0.0025	0.0027	0.0036	0.0042
Foll.	Tiny,few	Tiny,few	Tiny,few	Tiny,few	Tiny,few	Tiny,few
Position	Internal	Internal	Internal	Internal	Internal	Internal
C.L.	None	None	None	None	None	None
L.,Treated			Untreated			
Weight	.0025	.0031	.0027	.0177	.0240	.0317
Foll.	Big,few	Big,few	Tiny,few	Big,few ₁	Big,com. ₁	Big,com. ₁
Position	Subintern.	Internal	Internal	External	External	External
C.L.	None	None	None	Com.b.f.	Abun.b.f.	Abun.b.f.
Uter.Horns						
R.,Untreat.						
Weight	.0143	.0293	.0255	.0196	.0221	.0220
Situ	1.0	1.1	1.0	0.9	1.15	0.8
Activity	None	None	None	None	None	None
Endomet.	Low	Low	Low	Low	Low	Low
L.,Treated			Untreated			
Weight	.2725	.3825	.0215	.1295	.1694	.1735
Situ	3.8	3.8	1.0	2.8	3.0	2.65
Activity	Vasc.,mot.	Vasc.,mot.	None	Vasc.,mot.	Vasc.,mot.	Vasc.,mot.
Endomet.	Mod.high+	Mod.high+	Low	Mod.high	Mod.high	Mod.high
Vagina						
Orifice	Nov. 6	Nov. 7	Closed	Dec. 11	Dec. 11	Dec. 11
Smear	Abun. E	Abun. E	Prepub.	Abun. E	Abun. E	Abun. E
Corn.Cond.	Mod.high	Mod.high	Low	Mod.high	Mod.high	Very high
Weight				.4194	.3799	.6006

¹In females 277, 279, 282, large follicles quickly turn magenta, maroon, black, usually only smaller and more internal ones remaining typically clear; of larger follicles, some become cystic, others luteinize.

brought normal female ground-squirrels into apparently full breeding activity in a week's time. The vagina, previously closed tightly, opened in July, August, November, and December in an apparently normal fashion with the production of an oestral smear exceedingly abundant and thick, consisting of an unmixed mass of the large, enucleate cornified cells found normally only in late oestrus. The ovary, however, remained small--even decreased

TABLE VIII

REACTION TO OESTRIN APPLICATION

Animal No.	Treated 25	Treated 26	Control 30	Treated 293	Treated 294	Control 296
Source	Lab.	Lab.	Lab.	Lab.	Lab.	Lab.
Age	Ad.imm.	Ad.imm.	Ad.imm.	Ad.imm.	Ad.imm.	Ad.imm.
Condition	Prepub.low	Prepub.low	Prepub.low	Prepub.low	Prepub.low	Prepub.low
Operated	Oct. 27	Oct. 27	Oct. 27	Dec. 4	Dec. 4	Dec. 4
Spaying	Unilat.	Unilat.	Unilat.	Bilat.	Bilat.	Bilat.
Daily	12.5 R.U.	25 R.U.	Phys.sal.	1 R.U.	6 R.U.	Olive oil
Period	19 days	19 days	19 days	7 days	7 days	7 days
Posted	Nov. 18	Nov. 18	Nov. 18	Dec. 12	Dec. 12	Dec. 12
Ovaries						
R.,Cond.	Untreated	Untreated	Untreated			
Weight	.0041	.0026	.0025			
Foll.	Tiny,few	Tiny,few	Tiny,few			
Position	Internal	Internal	Internal			
C.L.	None	None	None			
L.,Cond.	Treated	Treated	Untreated			
Weight	.0029	.0033	.0027			
Foll.	Prac.none	Prac.none	Tiny,few			
Position	Internal	Internal	Internal			
C.L.	None	None	None			
Uter.Horns						
R.,Cond.	Untreated	Untreated	Untreated	Treated	Treated	Untreated
Weight	.0155	.0123	.0255	.1662	.4597	.0083
Situ	1.1	0.8	1.0	2.45	3.4	0.9
Activity	None	None	None	Vasc.,mot.	Vasc.,mot.	None
Endomet.	Low	Low	Low	Mod.high	High	Low
L.,Cond.						
Weight	.4479	.2175	.0215	.1707	.4295	.0093
Situ	4.3	3.6	1.0	2.45	3.4	0.9
Activity	Vasc.,mot.	Vasc.,mot.	None	Vasc.,mot.	Vasc.,mot.	None
Endomet.	Very high	Very high	Low	Mod.high	Mod.high	Low
Vagina						
Orifice	Nov. 6	Nov. 6	Closed	Dec. 12	Dec. 10	Closed
Smear	Enorm. E	Abun. E	Prepub.	Abun. eE	Abun. E	Prepub.
Corn.Cond.	Very high	Very high	Low	Quite high	Quite high	Quite low
Weight				.5642	.6279	.1441

slightly in size in one animal--and showed no signs of increase in activity. An oestral condition was conspicuous in uterine horn and vaginal tract.

Injections of oestrin following the removal of both ovaries gave exactly the same effect, (see females 293 and 294 in Table VIII) showing that the action of the oestrin is direct on the accessories and not through the ovary. All of the reproductive accessories were built up, including the nipples and the mammary glands. These experiments demonstrate that the reproductive accessories will react to oestrin at any time of the year.

4. Assay for Seasonal Variation in Potency of the Anterior Pituitary of the Ground-Squirrel

Having demonstrated that the ovary of the ground-squirrel-- as well as the entire genital tract--is susceptible to stimulation by the anterior pituitary hormone even at the period of apparent complete sexual inactivity, I next undertook to determine whether the ground-squirrel's own anterior pituitary is active and inactive at different seasons of the year and at different stages of the oestral cycle. This is made possible by the attenuation of the cycle where it would be almost impossible in a short-cycle mammal.

As a test of the potency of the anterior pituitary of the ground-squirrel, I used the immature female albino rat as an assay animal, the genital system of which reacts with striking rapidity to the gonad-stimulating hormone of the anterior pituitary by showing of marked sexual precocity. This precocity is evidenced by a tremendous enlargement of the ovaries, oviducts, uterus, and vagina; by rupture of the vaginal membrane and production of vaginal cornification (oestrus), easily detected by vaginal smears.

In these tests the dosage administered consisted of two anterior lobes each day for three successive days, taken from female ground-squirrels in the same reproductive condition, macerated and implanted into the leg muscles of immature female albino rats beginning on the twenty-first day of life. At the age of twenty-five days the rats were killed, together with littermate controls which had received no treatment; vaginal smears were taken and stained; and the reproductive organs were examined, measured, dissected out, and weighed. In first tests, the young rats were twenty-four days of age when tests were begun, but they showed the same response as those three days younger. Anterior pituitaries from 132 ground-squirrels were thus tested, in lots of six.

The anterior pituitaries of females which had just littered, June 1, gave very slight stimulation, almost none. About four weeks after littering, there was not the least sign of stimulation; this test was conducted around June 25. About October 15 I found that the anterior pituitaries from somatically adult females born in the field the previous spring were entirely impotent, producing not the least sign of stimulation. That these glands were in normal condition at such time and entirely able to produce the hormone, but simply were inactive, as well be shown below.

TABLE IX

SEX-STIMULATING POTENCY OF ANTERIOR PITUITARIES OF FEMALE GROUND-SQUIRRELS
WHEN IMPLANTED INTO IMMATURE FEMALE RATS

Treatment begun on 21st (*22nd, *24th) day of age,
2 lobes daily for 3 days, total 6 lobes;
animals posted 2 days later.

Rat No.	Ground-Squirrel				Total Dose	Rat Wt.	Ovs.* Ducts	Uter. Wt.	Ut.Horn Diams.	Vulva Opened	Smear
	Source	Age	Condition	Assayed							
32*	Field	Ad.	Lactating	Jun. 1-2	.0200	48	.0291	.0384	1.2x1.2	No	Pp
41*					None	49	.0237	.0297	1.2x1.2	No	Pp
44	Field ₁	Ad.	Postpart.	Jun.24-26	.0122	33	.0213	.0235	1.0x1.0	No	Pp
45					None	37	.0135	.0141	0.9x0.9	No	Pp
46	Lab.	Ad.	Spayed	Oct.12-14	.0110	41	.0584	.1804	3.2x2.8	Oct.15	e
49					None	38	.0196	.0212	1.0x1.0	No	Pp
50					None	38	.0226	.0208	1.1x1.0	No	Pp
53	Lab.	Imm.	Somat.ad.	Oct.12-14	.0098	39	.0204	.0168	1.1x0.9	No	Pp
54					None	54	.0198	.0218	1.2x1.0	No	Pp
57	Lab.	Ad.	Spayed	Oct.15-17	.0037	42	.0386	.0524	2.1x2.0	Oct.18	e
58	Lab.	Imm.	Somat.ad.	Oct.15-17	.0074	46	.0248	.0244	1.3x1.1	No	Pp
65	Field	Ad.	Prooest.	Apr.12-14	.0090	44	.0958	.0537	1.6x1.6	Apr.16	E
66	Lab.	Ad.	Spayed	Apr.12-14	.0151	40	.1510	.0683	2.8x2.6	Apr.16	E
68					None	42	.0247	.0237	1.1x1.0	No	Pp
69					None	39	.0217	.0239	1.1x1.1	No	Pp
72	Lab.	Ad.	Spayed	Apr.15-17	.0126	40	.0782	.0620	2.2x2.0	Apr.18	E
73	Field	Ad.	Prooest.	Apr.15-17	.0083	39	.0180	.0258	1.4x0.9	No	Pp
75					None	39	.0193	.0193	1.3x1.0	No	Pp
88°					None	55	.0245	.0298	1.2x0.9	No	Pp
89°	Field	Ad.	Midpreg.	May 14-16	.0123	55	.0361	.1751	3.1x2.8	Thin	Pe
90	Field ₂	Ad.	Oestrus	May 15-17	.0112	46	.0658	.1358	2.9x2.7	May 19	Pe
99	Field	Ad.	Lactating	Jun.18-20	.0133	43	.0211	.0231	1.3x1.0	No	Pp
101					None	40	.0186	.0218	1.2x0.9	No	Pp
103					None	49	.0241	.0278	1.5x0.9	No	Pp
104	Field	Ad.	Lactating	Jun.21-23	.0127	50	.0241	.0259	1.4x0.8	No	Pp
122	Field	Imm.	Age 3 mos	Aug.28-30	.0069	54	.0283	.0260	1.3x0.9	No	Pp

₁Captured afield and kept in laboratory 3 weeks.

₂Captured afield in prooestrus, kept in laboratory 2 weeks, removed to outdoor pens where full oestrus was assumed in a few days.

The anterior pituitaries of females caught in the field in a prooestral condition and tested immediately gave border-line results. One lot, in which the reproductive tracts were slightly smaller, gave no apparent stimulation; yet another lot tested at the same time, but which had reproductive tracts somewhat more active, gave a good showing of potency and stimulated an immature rat appreciably. In the latter case the ovaries and uteri of the test animal were both increased in weight about 300 per cent. See rat No. 65 in Table IX, and compare with rat No. 73.

Females still sexually active and virgin, segregated in outdoor cages and remaining in full oestrus to May 15, produced very good stimulation when their anterior pituitaries were tested in immature rats, increasing ovarian weights by 250 per cent and uteri by 400 per cent. There was also very good stimulation from anterior pituitary glands of May 15 females in normal mid-pregnancy, the test animal's ovaries being 160 per cent heavier and uteri 600 per cent.

Thus we found that the anterior pituitary of normal ground-squirrels from the field is quite active during April and May, when the animals are in Prooestrus, oestrus and pregnancy; but that immediately after littering the potency of these glands is very slight, while a month after littering they are completely inactive insofar as this type of test could determine.

5. Effects of Spaying on the Potency of the Anterior Pituitary of the Ground-Squirrel

I also determined by bioassay in immature female rats twenty-one days of age that the anterior pituitaries of sexually inactive female ground-squirrels show decided potency following ovariectomy. Normal animals in the laboratory in early fall, spayed approximately two and a half months after littering in the field, showed a high production of gonad-stimulating substance. The same was true of somatically adult immatures kept in the laboratory through fall and winter; unoperated females kept in the laboratory until April showed little activity, while those gonad-ectomized at eight months of age, showed a very high production of the gonadal stimulant when their anterior pituitaries were tested a couple of months later.

These experiments demonstrate that the animals are capable of producing gonad-stimulating substances in their anterior pituitaries in fall and early spring; but, what is more important from the standpoint of our problem, they indicate that the production of such gonad-stimulating hormones by the anterior pituitary

TABLE X

SEX-STIMULATING POTENCY OF BLOOD SERUM FROM FEMALE GROUND-SQUIRRELS
WHEN INJECTED INTO IMMATURE FEMALE RATS

Treatment: 1 cc. thrice daily for 5 (*3) days,
total 15 (*9) cc., begun on 21st day;
animals posted when 27 (*25) days old.

Rat No.	Ground-Squirrel		Blood Serum		Rat Wt.	Ovs.+ Ducts	Uter. Wt.	Ut.Horn Diams.	Vulva Opened	Smear
	Source	Condition	Taken	Assayed						
49*					38	.0196	.0212	1.0x1.0	No	Pp
50*					38	.0226	.0208	1.0x0.8	No	Pp
51*	Lab.	Ad.imm.	Oct.12-14	Oct.12-14	41	.0194	.0202	1.0x0.8	No	Pp
52*	Lab.	Spayed	Oct.12-14	Oct.12-14	31	.0232	.0174	1.0x0.7	No	Pp
59*	Lab.	Ad.imm.	Oct.15-17	Oct.15-17	41	.0238	.0196	1.0x0.7	No	Pp
60*	Lab.	Spayed	Oct.15-17	Oct.15-17	36	.0222	.0216	1.0x0.9	No	Pp
83	Field	Prooestrus	Apr.12-14	May 1-3	30	.0270	.1010	2.0x1.8	May 3	e
84	Lab.	Spayed	Apr.12-14	May 1-3	36	.0649	.1425	2.6x2.1	May 3	E
87					34	.0203	.0200	1.2x1.0	No	Pp
91					55	.0259	.0344	1.4x1.1	No	Pp
92 ₁	Field	Oestrus	May 15-17	May 16-20	45	.0192	.0213	1.1x0.9	No	Pp
93	Field	Midpreg.	May 14-16	May 16-20	46	.0225	.0290	1.3x0.8	No	Pp
94 ₂	Field	Prooestrus	Apr.15-17	Jun.12-14	33	.0182	.0270	1.3x1.0	No	Pp
95					45	.0211	.0239	1.4x1.0	No	Pp
96	Lab.	Spayed	Apr.15-17	Jun.12-16	46	.0664	.0946	2.5x1.3	June 16	E
101					40	.0186	.0218	1.2x0.9	No	Pp
102	Field	Lactating	Jun.18-20	Jun.18-22	26	.0146	.0340	1.8x0.6	No	Pp
123	Field	3 mo.imms.	Aug.28-30	Aug.28-30	48	.0304	.1123	2.3x1.5	Sept.2	E
124					70	.0239	.0219	1.2x0.8	No	Pp
125	Field	Lactating	June 21-23	Oct. 2-6	59	.0219	.0200	1.1x0.7	No	Pp
126	Field	4 mo.imms.	Oct. 2-5	Oct. 2-6	61	.0266	.0276	1.2x0.7	No	Pp
127					71	.0240	.0292	1.2x0.7	No	Pp
128					61	.0239	.0259	1.2x0.7	No	Pp
144					63	.0296	.0295	1.3x0.8	No	Pp
145	Lab.	Post-lact.	July 19	Nov. 4-8°	52	.0257	.0227	1.3x0.7	No	Pp
146	Field	Prooestrus	Apr.15-17	Nov. 4-8°	53	.0239	.0299	1.3x0.7	No	Pp

₁Captured afield April 9-20 in prooestrus; remained in prooestrus several weeks in laboratory, then in a few days in outdoor pens went into full oestrus.

₂Died on third day of injections, age 23 days.

°Small amount of serum, hence diluted N/5.

at such times is inhibited by the presence of the ovaries. While the ovary is not producing any obvious or detectable quantities of its hormones, yet it is still exerting some influence to keep the anterior pituitary repressed just as the spaying experiments previously discussed showed that it was maintaining the uterus and vaginal tract in a slightly above-castration, though apparently inactive, condition.

Further tests were made of the blood serum from animals used in these assays, as well as from those used in assays for activity in the normal pituitary of field animals. Again the immature female rat was used, though three days of treatment was found insufficient to detect activity in preliminary tests so that a longer period, five days of treatment, was later used in all tests. While fewer animals showed a potency reaction, these treatments gave contributory evidence to substantiate the finding and release of a gonad-stimulating substance from the anterior pituitary into the blood stream of the ground-squirrels.

6. Experimental Analysis of Environmental Factors

Seasonal changes in weather conditions and in the environment seem to have little or no effect on the reproductive rhythm in animals having shorter cycles--especially in laboratory rodents and other domesticated animals kept under more or less constant, artificial environmental conditions--but it is suggestive that in almost every case in nature the short period of oestrus or sexual activity in animals having a long annual cycle agrees well with seasonal changes and with periods of optimum environmental conditions, at least in temperate and colder regions. It therefore seemed advisable in the present study to try to determine the essential factor or factors controlling this coordination between environmental optimum and sexual activity.

In view of the fact that female ground-squirrels confined in the laboratory almost never come into reproductive activity, even when animals in the field are breeding, it was thought that perhaps food, light or darkness, heat or cold, or some similar factor varying in the field and laboratory might be a controlling element.

Food.--During each fall and winter the food of laboratory animals was constantly changed in an effort to permit normal sexual activity in female ground-squirrels. An abundance of fresh water, salt, and mixed grains was available at all times, and usually at least once a week fresh lettuce was given the animals on the chance that vitamin E might be involved. In November six

large cages were set up in the laboratory, each containing six animals so marked that their individual history could be followed through the winter. The feeding of these animals was as follows:

(a) Somatically adult, sexually immature females, given only wheat, oats, and barley.

(b) Somatically adult, sexually immature females, given wheat oats, barley, and corn.

(c) Somatically adult, sexually immature females, given a patented rabbit food certified to contain a balanced ration.

(d) Somatically adult, sexually immature females, given wheat, oats, and barley.

(e) Females somatically and sexually adult, given the rabbit food containing the balanced ration.

(f) Females somatically and sexually adult, given wheat, oats, barley, and corn.

Some of these animals were operated and inspected during February and March, but no precocious sexual activity was observed. Others were continually observed through the following spring and summer, yet they continued sexually as inactive as the general run of laboratory females.

Other females were kept continually lean by under-feeding or fat by over-feeding, yet no signs of sexual activity appeared.

Activity.--Several preliminary experiments were tried with activity cages. There was no tendency toward physical inactivity during the period of sexual inactivity; one female, for instance, kept her circular cage spinning, and on one occasion ran twenty-five miles in a single day. Furthermore, there seemed no stimulation toward sexual activity in animals physically active day after day for periods of several months. There was no apparent difference between the reproductive organs of such animals and the organs in almost completely inactive animals in the same laboratory.

Darkness.--Female ground-squirrels were kept for long periods of time during fall and winter in practically total darkness, some at ordinary room temperatures of about 70° Fahrenheit, others in a constant temperature room at 60°, yet not the slightest approach toward sexual activity was observed. Six months in cool darkness, where animals never went into full hibernation but only occasionally became cool and semi-torpid, showed no stimulation; neither did the return of animals to conditions of light and warmth after three months in such cool darkness.

Light.--On September 21, when the weather had become slightly cooler in the Chicago region and the length of day had shortened to an even twelve hours, various experiments were begun to determine whether light might be involved. At the start the

animals were given twelve hours of light a day, this being increased fifteen minutes daily until at the end of forty days they were getting twenty-two hours of light and only two of darkness. This last ratio was then maintained for three months. One group of females was kept in a control room where the temperature was maintained at a constant 60° Fahrenheit and the humidity at 70 to 80 per cent; illumination was entirely artificial, by use of ordinary frosted electric lights, so that part of the group got 80 foot-candles and another part 160 foot-candles. Another group was maintained in warmer hothouse temperatures of 70° to 80° Fahrenheit, with rather high humidity; these animals got all available natural sunlight through the ordinary window-glass that roofed the hothouse and walled it on east, south, and west; the added hours of artificial light were given in early morning and during the evening by means of frosted electric lights having the same power as those used in the control room.

In none of these animals was there any suggestion of stimulation. They remained as sexually inactive as animals kept in the laboratory colony or in darkness.

Cold Darkness.--The most striking effects were obtained with females kept in the cold room at Whitman Laboratory, at a constant 40° Fahrenheit and in complete darkness except during brief daily intervals when the light was snapped on for a quick inspection. Sixteen females, isolated under such conditions in midsummer and in fall, quickly went into full hibernation; and, though some were furnished only dry grain while others were given green vegetables and mixed grains and water, all went through alternating periods of activity and of hibernation, each lasting from a day to several weeks, and each female running a schedule of her own apparently unrelated to the hibernating of other females in the same room.

All of these females were in a state of complete sexual inactivity at the time they were introduced into the cold room, yet each went in an entirely normal fashion from a condition of anoestral or prepubertal rest through a normal prooestrus and into full oestrus--evidenced by a rich vaginal smear of cornified cells after establishment of the vaginal introitus, the great enlargement and hardening of the cervix and the establishment of highly cornified epithelium in the vagina, development of the uterus and its glands to an oestral condition, and enlargement of the ovary with development of Graafian follicles. This development occurred at about the same rate as in normal animals in nature, beginning with the time when they were put into the cold room. Immatures from the previous season, intro-

TABLE XI

EFFECTS OF APPLICATION OF CONSTANT COLD (40° F.) AND DARKNESS
TO SEXUALLY INACTIVE ADULTS

(All tissues show effects of treatment.)

No.	519*	686	690	696	562	560
Source	Lab.	Lab.	Lab.	Lab.	Lab.	Lab.
Age at Start	Adult	Immature	Adult	Adult	Adult	Adult
Condition	Regressed	Prepub. low	Regressed	Regressed	Receded	Receded
Previously	Pregnant	Virgin	Pregnant	Pregnant	Virgin	Virgin
Treatment						
Begun	Feb. 4	Nov. 14-20 ₁	July 15	July 15	Aug. 8	Aug. 8
Duration	148 days	384 days	147 days	211 days	124 days	188 days
Hibernated	60 days	93 days	31 days	125 days	73 days	94 days
Active	88 days	291 days	116 days	86 days	51 days	94 days
Posted	July 2*	Dec. 3	Dec. 10	Feb. 12	Dec. 10	Feb. 12
Ovaries						
R., Weight	.0079	.0059	.0056	.0040	.0061	.0077
Foll.	Med., few	Small, com.	Small, com.	Small, com.	Med., abun.	Large, abun.
Position	External	Subsurf.	Subsurf.	Surface	External	External
C.L.	None	None	None	None	None	None
L., Weight	.0080	.0046	.0063	.0049	.0060	.0069
Foll.	Med., few	Med., few	Small, com.	Small, com.	Med., abun.	Large, com.
Position	External	Surface	Subsurf.	Surface	External	External
C.L.	None	None	None	None	None	None
Uter. Horns						
Situ	3.75	2.73	2.88	3.30	3.38	3.28
R., Weight	.8000	.0937	.1157	.1480	.1102	.1809
Length	70.20	36.30	41.15	75.90	31.00	64.50
Diams.	5.25x2.90	2.35x1.50	2.47x1.70	2.20x1.41	2.40x1.98	2.35x2.20
L., Weight	.2925	.0915	.1591	.1473	.0970	.1576
Length	65.00	37.55	38.20	75.30	31.10	57.50
Diams.	2.70x2.40	2.40x1.50	2.85x1.82	2.00x1.60	2.40x2.00	2.48x2.06
Activity	Vasc., mot.	Vasc., mot.	Vasc., mot.	Vasc., mot.	Vasc., mot.	Vasc., mot.
Endomet.	Fair, high	Mod. high	Fair, high	Fair, high	Fair, high	Fair, high
Vagina						
Orifice						
At begin.	Closed	Closed	Closed	Closed	Closed	Closed
Opened	June 1	May 15	No	Jan. 30	Dec. 5	Dec. 20
Smear	Abun. E	Abun. E	Prepub.	Abun. E	Abun. E	Abun. E
Last Sm.	Abun. 1e+E	Pro+E+1e	Prepub.	Early E	Abun. E	Abun. E
Corn. Cond.	Mod. high	Low	Near	Quite high	Quite high	Quite high
Weight	1.5597	.7334	.3665	.8095	.8964	.8197

*Killed 24 hours after copulation; marked change occurred in vaginal smear, from abundant oestrus at copulation to mixed smear with strong infiltration of leucocytes at posting.

₁Put into 60° Fahrenheit on Nov. 14, then into 40° F. cold darkroom on Nov. 20, where it remained beyond time when it would normally pass through pubertal changes.

duced into cold darkness in November, came into full oestrus the following May; normal adult females which had littered the previous spring, introduced into the cold room in July, were in sexual activity by February. There was some indication that adult females kept virgin the previous spring, placed in the cold room in August, were in activity as early as December. Thus virgin females reacted quickest to this environment, females which had littered reacting somewhat slower, while the slowest were the somatically adult immatures which had not yet passed through a breeding season.

Another puzzling question arises when one learns that these females remained in a condition of full oestrus as long as they were kept in such cold dark conditions--one of them, female No. 686, shown with other typical animals in Table XI, remaining in oestrus for more than seven months, at the end of which time she was killed for recovery of tissues. Ovaries of these animals could not be distinguished from those killed as field controls during April; ovarian size and follicular activity were entirely comparable, and neither cystic follicles nor blood-follicles nor corpora lutea resulted from cold-room stimulation even under long-time treatment. Ovaries and accessories in long-time female No. 686 were found in a lower condition of activity, as evidenced both by weight and histological picture, than was expected; apparently she had receded somewhat from a high level of activity as a result of continued retention in cold darkness, and apparently reached a balanced lower level of hormone production. This is reflected in the infiltration of some leucocytes in the smear, the resumption of small nuclei in many of the cornified cells, and the disappearance of all cornified layers from certain parts of the vaginal lumen leaving a lower epithelium consisting of cuboidal and semi-squamous epithelium. During a period of several weeks following attainment of a full oestral condition in the cold dark-room, the level of oestrin production is apparently kept at a high level--if one may judge from the greater weight of uterus and vagina and by thicker endometrium and sub-mucosal layers, as well as by enormous height of cornified layers over the vaginal epithelium.

Hibernation.--Although no separation has yet been made of the effects of hibernation on the one hand and of cold without hibernation on the other, there is good indication among cold room females that those animals which underwent a greater proportionate amount of hibernation showed an attenuation of the process of going from low to a high condition of sexual activity. This would be expected, since all processes should be slowed down in hibernation. Animals with a greater proportion of activity, showing

rapid respiration and high temperature, apparently attained sexual activity quicker.

VIII. DISCUSSION

The question of the control of cyclic breeding activity in female mammals is an involved one, yet a careful consideration of an annual-breeding wild mammal such as the thirteen-striped ground-squirrel used in these investigations suggests certain factors and interrelationships involved in such a mechanism of control.

The female ground-squirrel is here demonstrated to be sexually active for only a brief period once a year, during late spring and early summer, and sexually inactive for the remaining nine or ten months of the year. During the active breeding season the female attains a single high peak of oestrus or heat, followed quickly by coitus and pregnancy, which results in a single litter each year. Regression quickly follows parturition and lactation, and the female remains at a low peak of sexual activity from June to December, at which time a very slow and gradual aggression begins, so that by the following April the animal approaches another period of sexual activity. These observations are substantiated by the work of Johnson and Wade (1931) and of Johnson, Foster, and Coco (1933) on laboratory animals of another race of the present species; and by the observations of Foster (1934) on some representatives of the present form in Wisconsin.

A further analysis of these normal annual events in such a wild female mammal has been possible by the use of experimental procedures developed largely on laboratory rodents principally during the past ten years.

Despite the fact that during the period of low sexual activity the accessory reproductive organs of the female ground-squirrel appear to be involuted and completely inactive, removal of both ovaries is apparently followed by a slightly greater state of involution. Unilateral ovariectomy during this period of low activity apparently has no effect on the state of the accessories, which continue as if the animal possessed both ovaries.

These accessories are capable of responding to stimulation at any time of the year, as shown by the introduction of such oestrogenic substances as oestrin and theelin into spayed females; involuted accessories are quickly brought into a state of reproductive activity, without showing any stimulating effect on the

ovary at any time. Injections of oestrin, however, seem to stop all follicular development in treated ovaries and preventing the appearance of follicular antra. Such an inhibitory effect is understandable in the light of a reciprocal relationship in which the ovarian hormone shuts down the anterior pituitary so that the ovary no longer receives sufficient gonad stimulant to permit normal processes to continue.

Just as the accessories of the ground-squirrel are capable of responding to proper stimulation at any time of the year, the ovary is also capable, and through the action of the ovary the accessories respond. Smith and Engle, in a classic piece of research (1927), successfully demonstrated by experiments on the pied laboratory rat and the albino mouse that the ovary is not a self-regulating mechanism but is under the control of the anterior pituitary gland or hypophysis. They were able to show that the anterior pituitary hormone acts directly on the ovary and through it has a developmental and regulatory action on the rest of the genital system. Zondek and Aschheim (1927), working independently in Germany, demonstrated this same pituitary-ovarian control in the laboratory mouse.

In the present investigation on the female ground-squirrel, by using Smith's method of introducing fresh, macerated anterior pituitaries, I have been able to demonstrate repeatedly that the apparently inactive ovary of the ground-squirrel can be quickly developed to a high degree of functional activity, following which the reproductive accessories respond. Development of the entire reproductive tract--including the ovary, oviduct, uterus, vaginal tract (with introitus and full vaginal smear), and mammary glands can thus be induced at will. Similar results were obtained in some fifty other cases by the use of other gonad-stimulating substances of pituitary or pituitary-like origin, including extracts of fresh and dried sheep pituitaries, macerated fresh anterior pituitaries of rats or guinea pigs, untreated urine from pregnant women, and urinary extracts such as antuitrin-S and hebin.

Thus the ovaries and reproductive accessories of the female ground-squirrel were found capable of stimulation at any time of the year. Gonad-stimulants of the anterior pituitary, showing no effects on accessories of bilaterally ovariectomized females, stimulate the ovaries to secretory activity; the ovaries in turn produce sufficient female sex hormone, oestrin, to bring the accessories into high activity. Apparently, therefore, the long annual period of sexual inactivity in this wild female mammal is merely due to the absence of the anterior pituitary hormone in the animal's body at such time of the year.

Using the immature female albino rat as a test animal, by Smith's (1926) technique I was able to detect differences in the potency of the anterior pituitary gland of the female ground-squirrels themselves at different times of the year. Females in prooestrus and full oestrus have anterior pituitaries containing a high degree of gonad-stimulating substance; females in mid-pregnancy likewise showed a high pituitary activity. At other times of the year, using sexually inactive female ground-squirrels, I was unable to demonstrate any anterior pituitary activity. Foster (1934) has made several preliminary tests at Wisconsin confirming these findings.

The stimulating effects of the anterior pituitary gland on the gonads now seem clearly demonstrated, not only in laboratory and domesticated forms but in a wild type of mammal. Another relationship of the pituitary and the gonads is not so well known--Moore's (1930) theory of a reciprocal effect of the gonad on the pituitary. His working hypothesis holds that a high production of the sex hormone by the gonad has an inhibiting effect on the anterior pituitary, causing that gland to reduce or even stop the production of its gonad-stimulating hormone; that in the absence of the stimulating influence of the pituitary, the gonads quickly cease producing the sex hormone, the diminution of which permits the anterior pituitary to become active once more and so stimulate the gonads to another spurt of activity. Moore and Price (1932) offer extensive proof for this theory, based principally on their experiments with male albino rats.

Substantiation of this theory is found in the work of Engle (1929), who demonstrated in the rat that the gonad-stimulating capacity of the anterior pituitary is greatly increased after castration. Evans and Simpson (1929) then showed that the greatest increase in potency occurs during the first two weeks after castration, with a slower increase to the eighth post-operative week, after which no increase was found. Severinghaus has more recently (1932) shown the same increase in potency of the anterior pituitary following removal of the gonads in the guinea pig, while Smith, Severinghaus, and Leonard (1933) have demonstrated a similar condition in the rabbit.

In the present connection I have been able to show that in the female ground-squirrel complete removal of both ovaries induces increased potency in the anterior pituitary gland, even during the low period in fall. The results of implanting such pituitaries, about two months after spaying, into immature female rats is very striking. I was also able to demonstrate that this is not merely a phenomenon of manufacture and retention

without release by testing the blood-serum of such spayed female ground-squirrels in other immature female rats and finding a similar highly potent substance present, demonstrating its release into the blood-stream.

This line of investigation indicates that the apparently inactive ovary of the female ground-squirrel during the long period of sexual inactivity is nevertheless exerting some influence to keep the anterior pituitary repressed. As previously indicated, there is also some evidence to suggest that while the ground-squirrel ovary is not producing any detectable quantities of its hormones it is still maintaining the uterus and vagina in a normal healthy, though inactive condition slightly above the condition found in spaying.

In the main, Moore's theory as to the reciprocal pituitary-gonadal hormone relationship seems sufficient to account for cyclic reproductive activity in the female. This is especially true for the almost clock-like regularity of species having shorter rhythmic periods, including all of those like the rat or mouse, with a cycle of four or five days; the guinea pig, fifteen days; and even the primates, man and the monkeys, twenty-eight days.

As the ovary becomes active, it stimulates the accessories to full oestrus; at the same time--if we accept Moore's theory and the accumulating lines of corroborative evidence--when its production of oestrin becomes sufficiently high, the oestrin inhibits the pituitary. When the ovary receives no more stimulating hormone from the anterior pituitary, follicles fail to develop, and the production of oestrin falls off or stops entirely. In the absence of sufficient oestrin, the uterus and vagina decrease in size as the animal goes first into metoestrus and then into dioestrus (the short period of breeding rest). This removal of oestrin from the blood stream permits the anterior pituitary to become active. The ovary is then stimulated once more; follicles begin to develop, oestrin is produced anew, and such oestrin brings the genital passages into prooestrus and then full oestrus. Thus cycle follows cycle in the rat and mouse in the absence of pregnancy. Either the lutein hormone, or the oestrogenic hormones of the placenta, in pregnancy hold the pituitary in check until parturition, following which the next short cycle immediately occurs.

In the guinea pig a short period of pseudo-pregnancy occurs after each ovulation; and the activity of the corpora lutea, persisting for about two weeks after ovulation, delays the onset of the next cycle. This relationship was early shown by the experiments of Loeb (1910).

We are now in a position to see whether Moore's explanation will fit the long-drawn cycle of an annual breeding type of mammal. In the female ground-squirrel the ovary slowly becomes active in December, perhaps earlier, though the action of oestrin on the reproductive accessories is not detectable with certainty until about the first of the year. Oestrin is produced in gradually increasing amounts during January, February, March, and April, to the peak of oestrus during the latter half of April. Mating is followed by ovulation, corpus luteum formation, implantation and pregnancy. The anterior pituitary remains active at least to mid-late pregnancy, but then apparently loses its potency at or shortly after parturition; this situation is demonstrated by assay of anterior pituitaries in immature rats, and is also indicated by the continued presence of medium and large-sized follicles in ground-squirrel ovaries throughout pregnancy and their absence from postpartum ovaries. Here we must suppose that the production of oestrin is not sufficient to shut off the pituitary, which becomes comparatively inactive at the time the corpora lutea begin to bulge so hugely from the surface of the ovaries. The formation and structure of the corpus luteum in the ground-squirrel has already been ably studied by Drips (1919) who as a result of careful cytological studies described three distinct phases in the life of the corpus luteum of this species: (1) the occurrence of numerous red granules in the luteal cells, increasing rapidly from their first formation in the burst follicles after ovulation until their greatest abundance at mid-pregnancy, with a sudden drop at parturition, though a few granules persist four weeks longer; (2) the occurrence of lipid droplets in the luteal cells, first appearing at mid-pregnancy, increasing more rapidly just after parturition, until their peak of abundance is reached six weeks after parturition, then disappearing in two more weeks (by the end of July); and (3) regression of the corpora lutea, requiring about four weeks. The cessation of anterior pituitary gonad-stimulating capacity, which we find occurs at about the time of parturition, coincides with the sudden increase in occurrence of lipid droplets in the corpora lutea, so that it may well be the lutein rather than the oestrin hormone of the ovary which functions in the ground-squirrel as was demonstrated by Loeb in the guinea pig. And yet, though the activity of the corpora lutea apparently disappears about August 1 and signs of the corpora are difficult to find after September 1, the anterior pituitary is still held in check for several months.

The question then arises, what factor releases the mechanism and permits the pituitary to become active once more? The

coordination between the cycle and seasons of the year makes it obvious that some environmental factor is involved. The experiments of Rowan (1925-1930) on the junco and of Bissonnette (1930-1932) on the starling, emphasize the importance of light as a conditioning factor in birds; the work of Bissonnette (1932) and Hill and Parkes (1933) with ferrets, and of Baker and Ranson (1933) with field mice, suggest that light may be a factor in mammals, though more recent work by Hill and Parkes (1934) indicates that light is by no means necessary in the ferret. Craig-Bennet (1931) has found light of no importance in his experimental work on fishes, but emphasizes the necessity for relative changes in temperature.

One would tend to exclude light as a possible factor in the control of these events in the ground-squirrel, since reproductive activity is well begun underground in the darkness of the winter burrow. However, a series of experiments was carried through, using artificial illumination of different intensities under various controlled environmental conditions, that failed to suggest the least stimulation. No effect was secured by gradually lengthening the light periods, from twelve to twenty-two hours, within a period of two months, or by continuing such excess light treatment for another three months.

Other experiments were conducted, with a view to test the effect of varying types of diet, or darkness, constant warm or cool temperatures, and similar manipulations, but the results were negative in that they did not induce development or activity of the female reproductive organs or accessories of the ground-squirrel during the period of low activity.

However, animals placed in a cold dark room were released from sexual inactivity and came into sexual activity in an entirely normal manner, long before the normal season for such activity. Yet the aggression toward sexual activity occurred at about the normal rate for these animals in nature. It appears likely, therefore, that the cold treatment at 40° Fahrenheit--perhaps through the lowering of metabolism in hibernation--acts by stopping the slight activity of the ovary, thus releasing the anterior pituitary to become active once more during the frequent periods of physical activity experienced by the ground-squirrel during its long season of hibernation below ground. In addition, hibernation apparently slows down the rate of aggression so that pituitary activity, and hence ovarian activity, may rise very slowly toward sexual activity the following spring.

It seems, therefore, that periodic annual breeding animals differ from laboratory and domesticated animals because of

differential activity on the part of the anterior pituitary gland, periodically active and inactive in the long-cycle form and practically continuously active in the short-cycle ones. Further, the ovary apparently possesses the ability to exert an inhibitory influence on the anterior pituitary, at least during the period of low sexual activity. We realize that there are still weak links in the chain of evidence, with a few inconsistencies that have not yet been brought in line, but the greater part of the evidence indicates that Moore's conception of a reciprocal relationship between ovary and pituitary apparently actually exists in the ground-squirrel as a representative annual breeding type of wild mammal. None of the evidence seems yet to deny such a conception. However, such reciprocal relationship cannot be the whole picture, since the environment seems to play an important part, with cold temperatures in the present species together with other environmental factors such as darkness, hibernation, and winter inactivity, acting as checks and controls on such mechanism to bring it into functional coordination with optimal environmental conditions in nature. The weak links and slight inconsistencies can be explained away by further theorizing, but it seems better to wait until further facts bring clarification. With this in view, the investigations are being actively continued at the present time.

IX. SUMMARY AND CONCLUSIONS

1. Some six hundred female thirteen-striped ground-squirrels, captured afield between April and November, were used in an investigation to determine factors controlling rhythmical reproductive periodicity in annual breeding types of mammals. Examination of ovaries and reproductive accessories from a hundred and fifty adults during this period, and of ten postpartum females dug from outdoor hibernation pens between December and March, established the occurrence of a short season of sexual activity in late spring and early summer, with complete sexual inactivity throughout the remainder of the year.

2. A single period of oestrus occurs, once each year, lasting only until coitus a few days after emergence from hibernation in late April. Segregated females, however, continue in oestrus for several weeks.

3. Gestation occupies four weeks, with delivery of litters from mid-May to early June, followed by lactation for four to six weeks.

4. The reproductive tract undergoes the greater part of

its involution and regression immediately after parturition, though regression of corpora lutea does not occur until after cessation of lactation and proceeds slowly during the remainder of summer. The whole tract remains essentially inactive during fall.

5. Toward the end of the year, while the females are in hibernation below ground, the ovaries begin to show signs of activity, followed by a slow gradual development of oviducts, uterus, vagina, and mammary glands toward their peak in spring. Older animals emerge in a more advanced state of reproductive activity than do those born the previous spring.

6. The vaginal orifice opens during early oestrus, during the latter half of April, and closes during late lactation about the end of June.

7. Striking changes occur in the cell content of the vaginal smear throughout the year, rather similar to those found in the rat. Oestrus is characterized by a marked cornification of all cells, with complete elimination of leucocytes and other cell types. A leucocytic invasion occurs within thirty-six hours after coitus with another wave of leucocytes immediately after parturition. A colloid-like fluid marks the smear during lactation, but gives way later to the sparse smear characteristic of the anoestral rest occupying most of the year--a few nucleated epithelial cells, occasional monocytes, and traces of mucus.

8. A large series of adults and of somatically adult immatures was used in an attempt to analyze by experimentation the factors controlling the annual reproductive cycle in the female ground-squirrel.

9. Despite the apparent state of inactivity of the ovary during anoestrus, and the involuted condition of the accessory reproductive organs during the sexually inactive season, it was found that after removal of both ovaries there apparently occurred a slightly greater state of involution. This suggests that even during such period of apparent sexual inactivity the ovaries exert at least a minimal effect on the reproductive accessories. No obvious compensatory hypertrophy was observed after unilateral ovariectomy.

10. The ovaries and female accessories were found capable of responding to stimulation at any time of the year.

a. Development of the ovary, oviduct, uterus, vaginal tract (with introitus and full oestral smear), and mammary glands was induced at will by the application of gonad-stimulating substances, such as fresh anterior pituitary implants from rats and

guinea pigs, untreated urine from pregnant women, such urinary extracts as antuitrin-S and hebin, and extracts from both fresh and dried sheep pituitary. No effect was obtained when spayed females were used.

b. Development of the accessories was induced in spayed females by the introduction of such oestrogenic substances as oestrin and theelin. The same results were obtained when unoperated or unilaterally ovariectomized females were used, but the ovary itself remained apparently unaffected.

11. Using immature female rats as test animals, it was found that anterior pituitaries of normal female ground-squirrels from the field during late prooestrus, oestrus, and mid-pregnancy contain a relatively high degree of gonad-stimulating substance. Those from females killed during the long period of sexual inactivity showed no physiologically detectable trace of gonad-stimulating hormone; such animals included lactating females as well as those taken later in the season.

12. Removal of both ovaries induced increased potency in the anterior pituitaries of female ground-squirrels, even during the low period. Blood-serum tests verified these findings, demonstrating that even at the low stage of their activity the ovaries exert some inhibiting influence on the anterior pituitary.

13. It is concluded that, as in various other animals, the anterior pituitary of the ground-squirrel is the regulator of reproduction, acting directly on the ovary, which produces the oestrin necessary to bring the reproductive accessories into a high state of activity. Inactivity in the anterior pituitary is followed by inactivity in the entire reproductive tract.

14. In a series of experiments undertaken to analyze the possible involvement of environmental factors, it was found that variations in diet, the use of darkness, activity, constant warm or cool temperatures, removal from brief periods of cold or hibernation into warmth, did not induce development of the reproductive system during the period of sexual inactivity.

15. The daily addition of light by means of electric lights--gradually lengthening daily light periods from twelve to twenty-two hours within forty days--failed to suggest stimulation of the reproductive system. Further continued subjection to light periods of twenty-two hours a day for three months also failed to produce observable effects.

16. Animals kept continually in a cold darkroom at 40° Fahrenheit, with more or less normal hibernation, exhibited sexual development at any time of the year; and periods of oestrus were thus induced and maintained for as long as seven months, at

times of the year when female ground-squirrels are normally sexually inactive.

17. It is further concluded that while the ovaries and reproductive accessories are under the control of the anterior pituitary, which in turn is influenced and apparently inhibited by the ovary, environmental factors--such as cold or hibernation, possibly both--are also involved in the control of reproductive periodicity in the female ground-squirrel.

X. BIBLIOGRAPHY

- Baker, John R., and Ranson, R. M. 1932 Factors affecting the breeding of the field mouse (Microtus agrestis). I. Light. Proc. Roy. Soc. Lond., ser. B, vol. 110, p. 313.
- Bissonnette, Thomas Hume 1930 Studies on the sexual cycle in birds. I. Sexual maturity, its modification and possible control in the European starling (Sturnus vulgaris). Am. Jour. Anat., vol. 45, no. 2, p. 289.
- _____ 1930 Studies on the sexual cycle in birds. III. The normal regressive changes in the testis of the European starling (Sturnus vulgaris) from May to November. Am. Jour. Anat., vol. 46, no. 3, p. 477.
- _____ 1930 Studies on the sexual cycle in birds. IV. Experimental modification of the sexual cycle in males of the European starling (Sturnus vulgaris) by changes in the daily period of illumination and of muscular work. Jour. Exper. Zool., vol. 58, p. 281.
- _____ 1931 Studies on the sexual cycle in birds. V. Effects of light of different intensities upon the testis activity of the European starling (Sturnus vulgaris). Physiol. Zool., vol. 4, no. 4, p. 542.
- _____ 1932 Studies on the sexual cycle in birds. VI. Effects of white, green, and red lights of equal luminous intensity on the testis activity of the European starling (Sturnus vulgaris). Physiol. Zool., vol. 5, no. 1, p. 92.
- _____ 1932 Modifications of mammalian sexual cycles; reactions of ferrets (Mustela putorius) of both sexes to electric light added after dark in November and December. Proc. Roy. Soc. Lond., ser. B, vol. 110, p. 322.
- Bissonnette, T. H., and Chapnick, Morton Herman 1930 Studies on the sexual cycle in birds. II. The normal progressive changes in the testis from November to May in the European starling (Sturnus vulgaris), an introduced, non-migratory bird. Am. Jour. Anat., vol. 45, no. 2, p. 307.

- Brouha, L., and Simonnet, H. 1931 Considerations sur le determinisme du cycle ovarien chez les mammiferes. Proc. Second Internat. Cong. for Sex Research (London), p. 339.
- Craig-Bennet, A. 1931 The reproductive cycle of the three-spined stickleback, Gasterosteus aculeatus, Linn. Phil. Trans. Roy. Soc. Lond., ser. B, vol. 219, p. 197.
- Deanesly, Ruth 1934 The reproductive processes of certain mammals. Part VI. The reproductive cycle of the female hedgehog. Phil. Trans. Roy. Soc. Lond., ser. B, vol. 223, p. 239.
- Deanesly, Ruth, and Parkes, A. S. 1933 The reproductive processes of certain mammals. Part IV. The oestrous cycle of the grey squirrel (Sciurus carolinensis). Phil. Trans. Roy. Soc. Lond., ser. B, vol. 222, p. 47.
- Drips, Della 1919 Studies on the ovary of the spermophile (Spermophilus citellus tridecemlineatus) with special reference to the corpus luteum. Am. Jour. Anat., vol. 25, no. 2, p. 117.
- Engle, E. T. 1929 The effect of daily transplants of the anterior lobe from gonadectomized rats on immature test animals. Am. Jour. Physiol., vol. 88, p. 101.
- Evans, H. M., and Simpson, M. E. 1929 A comparison of anterior hypophyseal implants from normal and gonadectomized animals with reference to their capacity to stimulate the immature ovary. Am. Jour. Physiol., vol. 89, p. 371.
- Poster, Mark A. 1934 The reproductive cycle in the female ground squirrel, Citellus tridecemlineatus (Mitchill). Am. Jour. Anat., vol. 54, no. 3, p. 487.
- Hartman, Carl G. 1923 The oestrous cycle in the opossum. Am. Jour. Anat., vol. 32, p. 353.
- Hill, Margaret, and Parkes, A. S. 1933 Studies on the hypophysectomized ferret. V. Effect of hypophysectomy on the response of the female ferret to additional illumination during anoestrus. Proc. Roy. Soc. Lond., ser. B, vol. 113, p. 537.
- _____ 1934 Effect of absence of light on the breeding season of the ferret. Proc. Roy. Soc. Lond., ser. B, vol. 115, p. 14.
- Johnson, George E., and Wade, Nelson J. 1931 Laboratory reproduction studies on the ground squirrel, Citellus tridecemlineatus pallidus, Allen. Biol. Bull., vol. 61, no. 1, p. 101.
- Johnson, George., Foster, Mark A., and Coco, Russell M. 1933 The sexual cycle of the thirteen-lined ground squirrel in the laboratory. Trans. Kans. Acad. Sci., vol. 36, p. 250.
- Long, Joseph A., and Evans, Herbert McLean 1922 The oestrous cycle in the rat and its associated phenomena. Memoirs Univ. Calif., vol. 6, p. 1.

- Moore, Carl R. 1931 A critique of sex hormone antagonism. Proc. Second Internat. Cong. for Sex Research (London), p. 293.
- Moore, Carl R., and Price, Dorothy. 1930 The question of sex hormone antagonism. Proc. Soc. Exper. Biol. and Med., vol. 28, no. 1, p. 38.
- _____ 1932 Gonad hormone functions, and the reciprocal influence between gonads and hypophysis with its bearing on the problem of sex-hormone antagonism. Am. Jour. Anat., vol. 50, no. 1, p. 13.
- Rowan, William 1925 Relation of light to bird migration and developmental changes. Nature, no. 2892, vol. 115, p. 494.
- _____ 1926 On photoperiodism, reproductive periodicity, and the annual migrations of birds and certain fishes. Proc. Bost. Soc. Nat. Hist., vol. 38, no. 6, p. 147.
- _____ 1928 Reproductive rhythm in birds. Nature, no. 3062, vol. 122, p. 11.
- _____ 1929 Experiments on bird migration. I. Manipulation of the reproductive cycle: seasonal histological changes in the gonads. Proc. Bost. Soc. Nat. Hist., vol. 39, no. 5, p. 151.
- _____ 1930 Experiments in bird migration. II. Reversed migration. Proc. Nat. Acad. Sci. Wash., vol. 16, no. 7, p. 520.
- Smith, Philip E. 1926 Hastening development of female genital system by daily homoplastic pituitary transplants. Proc. Soc. Exper. Biol. and Med., vol. 24, p. 131.
- Smith, Philip E., and Engle, Earl T. 1927 Experimental evidence regarding the role of the anterior pituitary in the development and regulation of the genital system. Am. Jour. Anat., vol. 40, no. 2, p. 159.
- Smith, Philip E., Severinghaus, A. E., and Leonard, S. L. 1933 The effect of castration upon the sex stimulating potency and the structure of the anterior pituitary in rabbits. Anat. Rec., vol. 57, no. 2, p. 177.
- Stockard, C. R., and Papanicolaou, G. N. 1917 The existence of a typical oestrous cycle in the guinea-pig with a study of its histological and physiological changes. Am. Jour. Anat., vol. 22, p. 225.
- Zondek, B., and Ascheim, S. 1927 Das Hormone des Hypophysenvorderlappens. Klin. Wchnschr., vol. 6, p. 248.

